High Levels of CXC Ligand 12/Stromal Cell–derived Factor 1 in Apical Lesions of Endodontic Origin Associated with Mast Cell Infiltration

Franco Cavalla, DDS, MS, Montserrat Reyes, MT,† Rolando Vernal, DDS, PhD,* Carla Álvarez,* Rodolfo Paredes, DVM, PhD,‡ Jocelyn García-Sesnich, Licenciado en Bioquímica,* Magdalena Infante, DDS,* Valeska Fariña,* Ignacio Barrón,* and Marcela Hernández, MSc, DDS, PhD*†*

Abstract

Introduction: CXC ligand 12/stromal-derived factor-1 (CXCL12/SDF-1) is a pleiotropic chemokine that regulates the influx of a wide range of leukocytes. The aim of this study was to characterize CXCL12/SDF-1 in apical lesions (ALs) of endodontic origin, with special emphasis in associated immune cell populations. **Methods:** In this case-control study, 29 individuals with chronic apical periodontitis and 21 healthy volunteers were enrolled. ALs and healthy periodontal ligament samples were obtained for tissue homogenization, immune Western blotting, and enzyme-linked immunosorbent assay to determine CXCL12/SDF-1 forms and levels. Anatomopathologic diagnosis, immunostaining for CXCL12/SDF-1, CD117-CXCL12/SDF-1, and toluidine blue were also performed to identify tissue and cell localization. Finally, a set of tissue samples were digested and analyzed by flow cytometry to identify CXCL12/SDF-1 in different immune cell populations. Data were analyzed with Stata v11 and WinDi 2.9 software, and significance was considered if $P < .05$. **Results:** CXCL12/SDF-1 was predominantly identified as monomers; levels of CXCL12/SDF-1 were significantly higher in ALs compared with controls, and it was primarily localized to inflammatory infiltrates. Expression of CXCL12/SDF-1 was colocalized to mast cells in tissue sections. Furthermore, $CD117^+$ mast cells were the second most frequent infiltrating cells and the main CXCL12/SDF-1 expressing cells, followed by $CD4^+$ lymphocytes, monocytes/macrophages, neutrophils, and dendritic cells. **Conclusions:** ALs of endodontic origin demonstrated higher levels of CXCL12/SDF-1 compared with controls. CXCL12/SDF-1 was identified in immune cell populations, whereas mast cells represented the major CXCL12/SDF-1 expressing cells, suggesting that this chemokine might play a central role in apical tissue destruction, most probably inducing persistent recruitment of immune cells, particularly of mast cells. *(J Endod 2013;39:1234–1239)*

Key Words

Apical periodontitis, CXCL12/SDF-1, mast cells

Untreated dental caries can lead to bacterial infection of the dental pulp and the root canal system. Chronic apical periodontitis (CAP) or asymptomatic apical periodontitis (1) is the most common consequence of this infection, and its hallmark is the formation of an apical lesion (AL) that results from infection-induced inflammation and destruction of the hard and soft apical tissues (1). Histologically, healthy periodontal ligament evolves into an apical granuloma, which in turn might progress to a radicular cyst (2).

CAP-associated tissue destruction is characterized by the persistent migration of leukocytes into the lesional tissues. The process of selective recruitment and activation of these cell types by cytokine and chemokine messages decoded by specific surface receptors is ultimately responsible for the destructive events occurring in the apical periodontium (3).

CXC ligand 12/stromal cell–derived factor 1 (CXCL12/SDF-1) is a low-molecular-weight chemokine that is constitutively expressed in most connective tissues. Its pleiotropic effects include chemotaxis, differentiation, and survival in a variety of leukocytic cell populations that express its major receptor CXCR4, such as hematopoietic cells, lymphocytes, monocytes, and mast cells. CXCL12/SDF-1 promotes peripheral blood leukocyte adhesion to the vascular endothelium and consequent transendothelial migration to target tissues (3–5).

CXCL12/SDF-1 and CXCR4 gene expression has been identified in human ALs (4), whereas increased CXCL12/SDF-1 gene expression has also been reported in an experimental model of bacterial-induced apical periodontitis (4, 6). The major immune cell populations reported in ALs correspond to lymphocytes, monocytes/macrophages, and neutrophils, whereas mast cells have been reported to a much lesser extent (7–11). Nevertheless, involvement of CXCL12/SDF-1 in the progression of human ALs and its potential interactions with effector cells are yet to be known.

Considering the complex reciprocal functional interactions that the pleiotropic chemokine CXCL12/SDF-1 can establish with the apical microenvironment and

From the *Laboratorio de Biología Periodontal, Facultad de Odontología, Universidad de Chile, Santiago; †Departmento de Patología, Facultad de Odontología, Universidad de Chile, Santiago; and ‡Escuela de Medicina Veterinaria, Facultad de Ecología y Recursos Naturales, Universidad Andres Bello, Santiago, Chile.

Supported by FONDECYT grants nos. 1090461, 1120139, and 11100298 and UNAB DI 56-11/R grant.

Address requests for reprints to Dr Marcela Hernández, Laboratorio de Biología Periodontal, Facultad de Odontología, Universidad de Chile, Sergio Livingstone 943, Independencia, Santiago, Chile. E-mail address: mhernandezrios@gmail.com

0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2013.06.020

particularly with immune cells, the aim of this study was to characterize CXCL12/SDF-1 in ALs of endodontic origin, with special emphasis in associated immune cell populations.

Methods

Patients consulting at the clinics of diagnosis and endodontics, School of Dentistry, University of Chile were enrolled if they had clinical diagnosis of CAP as previously described (1), including the presence of an AL (compatible with an apical granuloma or cyst) detected by apical radiograph (>3-mm diameter) caused by dental caries in teeth with clinical diagnosis of nonvital pulp. Healthy periodontal ligaments from teeth extracted for orthodontic reasons were used as controls. Exclusion criteria included systemic illness or previous antibiotics or nonsteroid anti-inflammatory treatment during the 6-month period before the study. All the protocols and procedures were approved by the Ethics Committee of the School of Dentistry of the University of Chile and were in accordance with the ethical standards of the Declaration of Helsinki. An informed consent was obtained from all participating individuals or corresponding forms for their legal guardians in case of underage patients. The AL group was composed of 29 individuals, and the H group was composed of 21 individuals.

After tooth extraction, the AL and healthy periodontal ligaments (H) were frozen for subsequent homogenization, fixed in buffered formaldehyde at 4% for histologic procedures, or processed for tissue enzymatic digestion and flow cytometry.

Tissue Homogenates

After thawing, AL (n = 19) and H tissues (n = 18) were weighed, and protein extracts were obtained by automated homogenization in 50 mmol/L Tris-HCl pH 7.5, 0.2 mol/L NaCl, 5 mmol/L $CaCl_2$, and 0.01% Triton X-100 buffer adding EDTA-free proteinase inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany) in a constant ratio of 10 μL buffer per mg weighed tissue, centrifuged at 13,000*g* for 6 minutes at 4°C, and stored at −80°C until further analysis by immuno Western blotting and enzyme-linked immunosorbent assay.

CXCL12/SDF-1 Western Blot

A sodium dodecylsulfate–polyacrylamide gel electrophoresis in a 15% gel was performed in aliquots containing 20 μg tissue homogenates and transferred onto a nitrocellulose membrane (Pierce Biotechnology, Rockford, IL). For Western hybridization, the membrane was blocked with 3% bovine serum albumin in Tris buffered saline–Tween 0.1% (TBS-T) for 1 hour at room temperature, and then the membrane was incubated for 1 hour with a primary monoclonal antibody against CXCL12/SDF-1 (R&D Systems, Minneapolis, MN) in a 1:125 dilution on blocking solution. The membrane was washed 3 times with TBS-T and subsequently incubated for 1 hour with a 1:6000 dilution of a goat anti-mouse horseradish peroxidase–conjugated secondary antibody (Pierce Biotechnology) and then washed 3 times with TBS-T. Images of the hybridized bands were acquired with an enhanced chemiluminescence detection kit ECL (Pierce Biotechnology) in a Gel Logic 212 Pro imaging system (Carestream, Rochester, NY).

CXCL12/SDF-1 Levels

CXCL12/SDF-1 levels were measured in tissue homogenate samples by using a Quantikine ELISA human CXCL12/SDF-1α immunoassay (R&D Systems), following the manufacturer's instructions.

Histologic Procedures

Tissue samples were included in paraffin and routine processing for diagnosis of apical granuloma (n = 3), radicular cyst (n = 3), or healthy periodontal ligament (n = 3). CXCL12/SDF-1 was immunolocalized in tissue sections by immunohistochemistry, and positive cells were first identified on the basis of morphologic criteria. To confirm the identity of CXCL12/SDF-1–immunopositive cells, toluidine blue dye and CXCL12/SDF-1 and CD 117/KIT (mast cells) double immunofluorescence procedures were performed.

Immunohistochemistry

Tissue sections of 4 micrometers were cut and deparaffinized, and endogenous peroxidase activity was quenched by incubation in 10% hydrogen peroxide. Antigen retrieval was performed with Proteinase K, following manufacturer's recommendations (Novocastra; Lab. Novo, Newcastle, UK), and unspecific blocking was performed with 2.5% horse serum for 10 minutes (Kit ABC Universal Vectastatin; Vector Laboratories, Burlingame, CA). Monoclonal primary antibody against human CXCL12/SDF-1 (R&D Systems) was incubated overnight in 1:60 dilution and rinsed. The immunostaining was performed with Vectastatin Elite ABC kit (Vector Laboratories) by using anti-mouse biotinylated secondary antibody, developed with DAB (Zymed Labs Inc, San Francisco, CA), counterstained with Mayer's hematoxylin (Merck KGaA, Darmstadt, Germany), and mounted. Slides were examined by using an optical microscope (Zeiss Axiostar Plus, Poughkeepsie, NY), and representative images were acquired by using a microscope-mounted digital camera (Axiocam ERc5s; Zeiss, Göttingen, Germany). Positive and negative controls were processed within each series.

Toluidine Blue Staining

Mast cells were identified in tissue sections by toluidine blue dye. Toluidine blue working solution was prepared by mixing 5 mL toluidine blue dye (Sigma-Aldrich, St Louis, MO) with 45 mL 1% sodium chloride and adjusted to pH 2.3 with glacial acetic acid (Merck KGaA). Deparaffinized sections of 5-μm thickness were stained in toluidine blue working solution for 2–3 minutes at room temperature, washed in distilled water 3 times; dehydrated in 95% and 100% alcohol, cleared in xylene for 3 minutes, and covered with coverslips and resinous mounting medium. Mast cells were identified by their characteristic violet metachromasia in an optical microscope (Zeiss Axiostar Plus), and representative images were acquired by using a microscope-mounted digital camera (Axiocam ERc5s).

Double Immunofluorescence

Tissue sections of 4 micrometers were cut and deparaffinized. Antigen retrieval was performed with citrate buffer pH 6 in a microwave oven for 20 minutes. The autofluorescence was blocked with glycine 0.1 mol/L for 10 minutes, and unspecific protein blocking was performed with Pro-Block (ScyTek Laboratories, Logan, UT), following the manufacturer's instructions. A polyclonal primary anti-human CD117/KIT antibody (Thermoscientific, Asheville, NC) was incubated for 1 hour in 1:100 dilution and rinsed with phosphate-buffered saline (PBS). A second monoclonal anti-human CXCL12/SDF-1 antibody (R&D Systems, Inc) was incubated overnight in 1:50 dilution and rinsed with PBS-1x. The slides were subsequently incubated with appropriate secondary antibodies Alexa546 anti-rabbit and Alexa488 anti-mouse (Invitrogen, Carlsbad, CA), respectively, for 1 hour and mounted in Vector Vectashield mounting medium containing DAPI (Vector Laboratories), covered, and sealed. Slides were examined by using an epifluorescence microscope (Olympus BX41, Center Valley, PA), and representative images were captured with a microscope-mounted

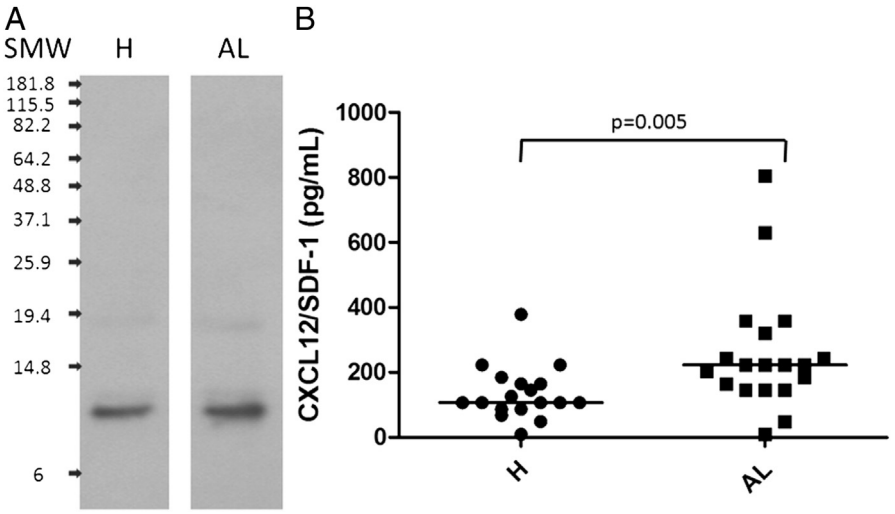

Figure 1. CXCL12/SDF-1 immunoreactive forms (*A*) and levels (*B*) in apical lesions (ALs) and healthy periodontal ligament. H, healthy periodontal ligament; SMW, standard of molecular weight. *Bars* represent medians.

digital camera (MicroPublisher 3.3 RTV QImaging, Surray, BC, Canada). Digital images were then processed with an image editing software (QCapture Pro v6.0, Surray, BC, Canada).

Tissue Enzymatic Digestion

ALs (n = 4) were processed by enzymatic digestion to obtain a total cell suspension as previously described (12). Briefly, samples were minced into approximately 1-mm^3 pieces and incubated in tissue digestion medium at 37°C for 90 minutes. The tissue digestion medium consisted of RPMI1640 supplemented with 50 IU/mL penicillin, 50 μg/mL streptomycin, and 200 mmol/L L-glutamine, plus 200 U/mL type IV collagenase (Gibco Invitrogen Corp, Grand Island, NY). Cells were then washed twice with PBS; cell counting was performed in a Neubauer chamber by using a phase-contrast microscope (Axiovert 100; Zeiss Co), and cell viability (≥90%) was calculated by trypan blue dye exclusion.

Flow Cytometry Analysis of CXCL12 Expression

Fifty microliters PBS containing 2×10^5 total cells of each sample were incubated separately with 10 μL fluorescein isothiocyanate (FITC)–conjugated anti-CXCL12/SDF-1 monoclonal antibody and 10 μL of the following phycoerythrin (PE)-conjugated monoclonal antibodies: anti-CD1a (matured dendritic cells), anti-CD4 (helper T lymphocytes), anti-CD8 (cytotoxic T lymphocytes), anti-CD14

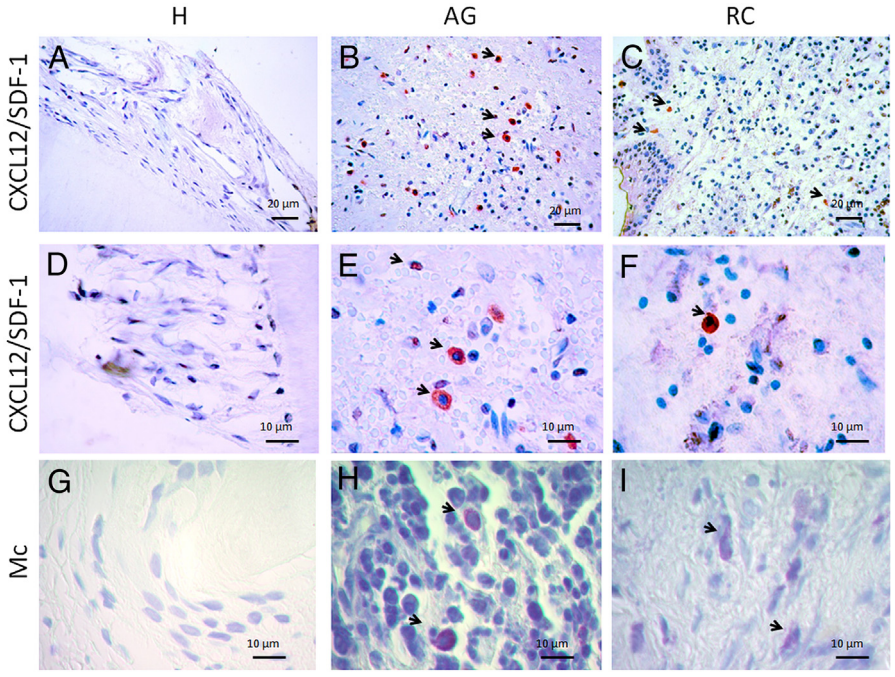

Figure 2. Tissue localization of CXCL12/SDF-1 (*A–F*) and mast cell identification (*G–I*) in apical lesions and healthy periodontal ligament. AG, apical granuloma; H, healthy periodontal ligament; RC, radicular cyst. *Arrows* represent immunopositive CXCL12/SDF-1 cells (*A–F*) and metachromatic mast cells (*G–I*).

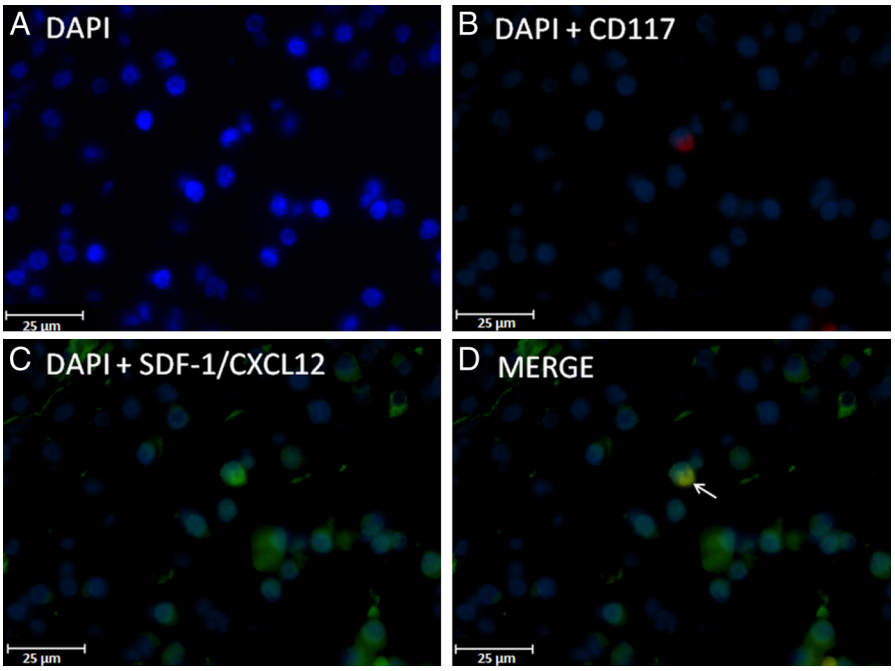

Figure 3. Colocalization of CD117 and CXCL12/SDF-1. (*A*) DAPI (nuclei); (*B*) DAPI + CD117 (mast cells); (*C*) DAPI + CXCL12/ SDF-1; (*D*) merge. *Arrow* indicates colocalization of CD117 and CXCL12/SDF-1.

(monocytes/macrophages), anti-16b (neutrophils), anti-CD19 (B lymphocytes), anti-CD56 (natural killer cells), anti-CD84 (activated dendritic cells), and CD117 (mast cells) for 30 minutes at 4°C in the dark (BD Biosciences Pharmingen, San Jose, CA). Cells were washed once in PBS and resuspended in 300 μL PBS to be analyzed by flow cytometry (Becton Dickinson, Franklin Lakes, NJ). Cells were gated according their forward- and side-scatter characteristics, and FITC-conjugated and PE-conjugated isotype-matched control monoclonal antibodies were used to determine the negative population.

Data Analysis

CXCL12/SDF-1 levels in tissue homogenates were analized by Shapiro-Wilk normality test and Mann-Whitney test using STATA v11 software (StataCorp, College Station, TX). Flow cytometry data were analyzed by using the WinMDi 2.9 software (Scripps Research Institute, La Jolla, CA) and represented as dot plots. CXCL12-positive cells were expressed in percentages. Statistical significance was considered if $P < .05$.

Results

The AL group (n = 29) was composed of 10 female patients with a mean age of 49.8 ± 11.7 years, whereas the H group (n = 21) was composed of 9 female subjects with a mean age of 12.9 ± 3.4 years. Significant differences were found for age ($P < .05$).

CXCL12/SDF-1 was identified through immune Western blotting in tissue homogenates from both healthy periodontal ligament and ALs, but it was more prominent in the latter. Immunoreactive bands consisted of ~9 kDa and faint ~18 kDa forms (Fig. 1*A*). Accordingly, CXCL12/SDF-1 levels were significantly higher in ALs when compared with healthy periodontal ligament ($P < .05$) (Fig. 1*B*).

Analysis of the immunostaining pattern of CXCL12/SDF-1 in ALs and healthy periodontal ligament revealed that the chemokine was mainly localized to inflammatory infiltrate in apical granulomas and radicular cysts (Fig. 2*B, C, E,* and *F*), whereas no detection was observed in healthy periodontal ligament (Fig. 2*A* and *D*). Strongly immunopositive leukocytes were identified on the basis of cell morphology as mast cells (Fig. 2*E* and *F*). Because mast cells can be clearly visualized by metachromasia of their granules, toluidine blue staining was performed to confirm the presence of mast cells in tissue sections (Fig. 2*G–I*). In a similar manner to SDF-1/CXCL12 staining pattern, mast cells were restricted to ALs in close relationship to inflammatory infiltrate, as well as in fibrous connective tissue. Finally, CXCL12/SDF-1 expression in mast cells was confirmed by double immunofluorescence of CXCL12/SDF-1-CD 117 (Fig. 3).

To further understand the association of CXCL12/SDF-1 and inflammatory cell populations in ALs and particularly with mast cells, biparametric analyses of CXCL12/SDF-1 and CD cell marker expression were quantified by flow cytometry (Fig. 4). Lymphocytes were the main cell population with 49.57% from total cells. Among them, 22.43% corresponded to helper T cells (CD4), 7.48% were cytotoxic T cells (CD8), 13.67% were B cells (CD19), and 5.99% were natural killer cells (CD56). Mast cells (CD117) represented the second most frequent cell population, corresponding to 31.42% of total cells. Monocytes/macrophages (CD14) were 13.67%, neutrophils (CD16b) were 9.78%, and dendritic cells (CD1a and CD83) were 8.45%, and 6.81% of them were activated dendritic cells (CD83). Total isolated cells expressing CXCL12/SDF-1 were 67.80%, and 31.02% of this expression was associated with mast cells ($P < .05$). Of note, almost 100% of mast cells expressed CXCL12/SDF-1.

Discussion

ALs of endodontic origin are characterized by persistent inflammation that results in the destruction of the apical periodontium and its replacement by granulation tissue in affected teeth. CXCL12/SDF-1 is a pleiotropic chemokine that functionally interacts with a wide variety of leukocytes, mainly via activation of CXCR4 receptor, regulating hematopoiesis, cell trafficking, and osteoclastogenesis (13,14).

The present study demonstrated higher levels of CXCL12/SDF-1 in ALs in comparison to its physiological counterpart the healthy

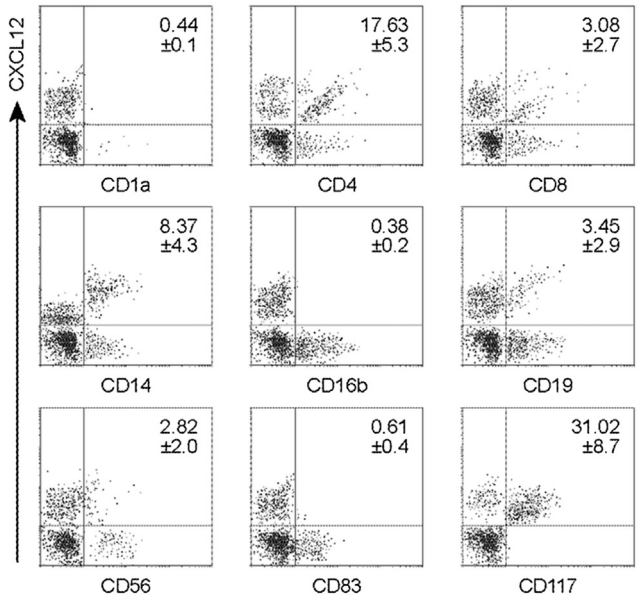

Figure 4. Flow cytometry analysis demonstrating CXCL12/SDF-1 expression by immune cells obtained from apical lesions. Representative dot plots for CXCL12/SDF-1 expression by matured dendritic cells (CD1a), helper T lymphocytes (CD4), cytotoxic T lymphocytes (CD8), monocytes/macrophages (CD14), neutrophils (CD16b), B lymphocytes (CD19), natural killer cells (CD56), activated dendritic cells (CD83), and mast cells (CD117) obtained from apical lesions. Data from each experiment were expressed as percentage of CXCL12/SDF-1 positive cells and represented as mean ± standard deviation from 4 independent experiments.

periodontal ligament, supporting a role of CXCL12/SDF-1 in progression of human CAP. Furthermore, the chemokine was identified in most of the infiltrating immune cell populations, including lymphocytes, monocytes/macrophages, neutrophils, and dendritic cells, whereas mast cells represented the major CXCL12/SDF-1 expressing cells.

CXCL12/SDF-1 was identified in ALs and healthy periodontal ligaments as 8.8 kDa monomeric forms and faint 19 kDa dimers. These forms are similar to those previously identified in gingival crevicular fluid and serum (5). It has been proposed that binding of monomeric CXCL-12/SDF-1 to CXCR4 receptor in the target cell fully activates chemotactic signaling pathways, whereas the dimeric form prevents cell migration, acting as an antagonist (15). On the basis of these findings, CXCL-12/SDF-1 in apical tissues should be seen predominantly as a functional chemoattractant agonist.

ALs exhibited higher protein levels of CXCL12/SDF-1 compared with healthy periodontal ligament. A drawback of periodontal ligament as a control is the age difference between diseased and healthy groups, because the latter are obtained for orthodontic purposes mostly in young patients; nevertheless, periodontal ligament corresponds to the closest histophysiological counterpart in humans. In line with these results, a basal production of CXCL12/SDF-1 has been described in periodontal ligament fibroblasts, suggesting a role in periodontal ligament homeostasis (16). Similarly, expression of both CXCL12/SDF-1 and its receptor CXCR4 has been identified in human dental pulp cells and stem cells, where they have been proposed to participate in their recruitment to the sites of injury (17, 18). Despite the few studies addressing CXCL12/SDF-1 in apical periodontitis, the current results are supported by Fukada et al (4), who identified CXCL12/SDF-1 and CXCR4 gene expression in human radicular cysts and apical granulomas and higher CXCL12/SDF-1 expression in both types of human ALs compared with healthy gingival tissue. Similarly, higher CXCL12/SDF-1 along with receptor activator of nuclear factor kappa B ligand, tartrate resistant acid phosphatase (TRAP)-positive osteoclasts, and bone resorption were identified in an experimentally induced apical periodontitis model in inducible macrophage-type nitric oxide synthase–deficient mice compared with their wild-type counterparts and healthy periodontal tissues (6), pointing to a pivotal role of CXCL12/SDF-1 over osteoclast recruitment/differentiation and bone resorption (13). Similar chemokine increased levels have been reported in marginal periodontitis in humans and animal models (19–21). Altogether, these results support a role for CXCL12/SDF-1 in apical periodontitis progression.

CXCL12/SDF-1 was identified in 67.80% of the total cells isolated from ALs, and all the immune cell populations analyzed were positive for the chemokine, including TCD4+, TCD8+, B and natural killer lymphocytes, monocytes/macrophages, neutrophils, activated dendritic cells, and mast cells. Inflammatory cells are essentially protective, but they can also cause severe damage to periodontal tissues on persistent and unregulated activation (22). It has been well established that CXCL12/SDF-1 exerts proinflammatory properties attracting monocytes/macrophages, T cells, early B-cell precursors, and neutrophils to peripheral tissues and acting as a costimulator of T-cell activation (23). Recently, it has been proposed that CXCL12/SDF-1 can skew the polarization of antigen-specific Tregs and interleukin-10 producing dendritic and monocytic cells under severe inflammatory conditions (23). Accordingly, the imbalance of immune and osteoclastic activities in ALs seems to be critically regulated by Treg cells (4). Whether CXCL12/SDF-1 participates in interleukin-10 release and Tregs commitment in ALs needs to be further explored.

Mast cell identification was restricted to ALs in a similar manner to CXCL12/SDF-1, in association with inflammatory infiltrates and connective tissue. Strikingly, mast cells represented the second major cell population from the total isolated cells after lymphocytes, as well as the main CXCL12/SDF-1 expressing cells, followed by specific CD4+ lymphocyte subpopulation and monocytes/macrophages. Mast cells are mobile, bone-marrow–derived, granule-containing immune cells that are found in all connective tissues. Previous studies that were based on immunohistochemistry have reported that mast cells were a far less abundant cell type in these kinds of lesions (24). It has been reported that most ALs had null to mild mast cell infiltration (10). Differences might be explained by the different methods employed; nevertheless, it cannot be excluded that the presence and role of mast cells in ALs might be underestimated. The cytoplasmic granules of mast cells contain a vast diversity of inflammatory mediators and catalytic enzymes that can play important roles in both direct tissue damage and inflammatory and immune regulation (7), including the selective recruitment of differential leukocyte subpopulations to the AL, bone metabolism, vascular phenomena, infection control, and hard and soft tissue repair (10, 25, 26).

In the present study we were able to colocalize CXCL12/SDF-1 and CD117 in AL. Although the nature of this association in apical periodontitis is unknown, mature mast cells as well as their blood precursors express CXCR4 (27). Recent evidence suggests that mast cells could be selectively chemoattracted through the CXCL12/SDF-1 CXCR4 axis in diverse inflammatory and tumourous diseases, such as allergic airway disease and glioblastoma multiforme (15, 28). Furthermore, on the basis of the finding that CXCL12/SDF-1 was identified in nearly all CD117+ cells, this might represent a major axis targeting mast cell migration to ALs. In addition, a synergistic effect over mast cell chemotaxis has been reported between CXCL12/SDF-1 and mast cell–derived histamine that was not observed for the other mast cell chemokines (16).

Conclusions

The present study demonstrated higher levels of CXCL12/SDF-1 in ALs of endodontic origin in comparison to healthy periodontal ligament, supporting a role of CXCL12/SDF-1 in progression of human ALs of endodontic origin. CXCL12/SDF-1 was identified in most infiltrating immune cell populations. Among them, mast cells represented the major CXCL12/SDF-1 expressing cells, suggesting that this chemokine might provide a central axis for sustained immune cell infiltration in ALs, particularly of mast cells.

Acknowledgments

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

References

1. Gutmann JL, Baumgartner JC, Gluskin AH, et al. Identify and define all diagnostic terms for periapical/periradicular health and disease states. J Endod 2009;35:1658–74.
2. Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med 2004;15:348–81.
3. Silva TA, Garlet GP, Fukada SY, et al. Chemokines in oral inflammatory diseases: apical periodontitis and periodontal disease. J Dent Res 2007;86:306–19.
4. Fukada SY, Silva TA, Garlet GP, et al. Factors involved in the T helper type 1 and type 2 cell commitment and osteoclast regulation in inflammatory apical diseases. Oral Microbiol Immunol 2009;24:25–31.
5. Villalba S, Salvucci O, Aoki Y, et al. Serum inactivation contributes to the failure of stromal-derived factor-1 to block HIV-I infection *in vivo*. J Leukoc Biol 2003;74:880–8.
6. Fukada SY, Silva TA, Saconato IF, et al. iNOS-derived nitric oxide modulates infection-stimulated bone loss. J Dent Res 2008;87:1155–9.
7. Marcal JR, Samuel RO, Fernandes D, et al. T-helper cell type 17/regulatory T-cell immunoregulatory balance in human radicular cysts and periapical granulomas. J Endod 2010;36:995–9.
8. Rodini CO, Lara VS. Study of the expression of CD68+ macrophages and CD8+ T cells in human granulomas and periapical cysts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;92:221–7.
9. Wang CY, Stashenko P. Kinetics of bone-resorbing activity in developing periapical lesions. J Dent Res 1991;70:1362–6.
10. Drazic R, Sopta J, Minic AJ. Mast cells in periapical lesions: potential role in their pathogenesis. J Oral Pathol Med 2010;39:257–62.
11. Walsh LJ. Mast cells and oral inflammation. Crit Rev Oral Biol Med 2003;14:188–98.
12. Vernal R, Dutzan N, Chaparro A, et al. Levels of interleukin-17 in gingival crevicular fluid and in supernatants of cellular cultures of gingival tissue from patients with chronic periodontitis. J Clin Periodontol 2005;32:383–9.
13. Wright LM, Maloney W, Yu X, et al. Stromal cell-derived factor-1 binding to its chemokine receptor CXCR4 on precursor cells promotes the chemotactic recruitment, development and survival of human osteoclasts. Bone 2005;36:840–53.
14. Veldkamp CT, Peterson FC, Pelzek AJ, Volkman BF. The monomer-dimer equilibrium of stromal cell-derived factor-1 (CXCL 12) is altered by pH, phosphate, sulfate, and heparin. Protein Sci 2005;14:1071–81.
15. Veldkamp CT, Ziarek JJ, Su J, et al. Monomeric structure of the cardioprotective chemokine SDF-1/CXCL12. Protein Sci 2009;18:1359–69.
16. Godot V, Arock M, Garcia G, et al. H4 histamine receptor mediates optimal migration of mast cell precursors to CXCL12. J Allergy Clin Immunol 2007;120:827–34.
17. Jiang L, Peng WW, Li LF, et al. Isolation and identification of CXCR4-positive cells from human dental pulp cells. J Endod 2012;38:791–5.
18. Jiang L, Zhu YQ, Du R, et al. The expression and role of stromal cell-derived factor-1alpha-CXCR4 axis in human dental pulp. J Endod 2008;34:939–44.
19. Havens AM, Chiu E, Taba M, et al. Stromal-derived factor-1alpha (CXCL12) levels increase in periodontal disease. J Periodontol 2008;79:845–53.
20. Abe D, Kubota T, Morozumi T, et al. Altered gene expression in leukocyte transendothelial migration and cell communication pathways in periodontitis-affected gingival tissues. J Periodontal Res 2011;46:345–53.
21. Hernandez M, Gamonal J, Salo T, et al. Reduced expression of lipopolysaccharide-induced CXC chemokine in Porphyromonas gingivalis-induced experimental periodontitis in matrix metalloproteinase-8 null mice. J Periodontal Res 2011;46:58–66.
22. Hernandez M, Dutzan N, Garcia-Sesnich J, et al. Host-pathogen interactions in progressive chronic periodontitis. J Dent Res 2011;90:1164–70.
23. Karin N. The multiple faces of CXCL12 (SDF-1alpha) in the regulation of immunity during health and disease. J Leukoc Biol 2010;88:463–73.
24. Yanagisawa S. Pathologic study of periapical lesions 1: periapical granulomas—clinical, histopathologic and immunohistopathologic studies. J Oral Pathol 1980;9:288–300.
25. Lima SC, Rizo VH, Silva-Sousa YT, et al. Immunohistochemical evaluation of angiogenesis and tryptase-positive mast cell infiltration in periapical lesions. J Endod 2011;37:1642–6.
26. de Oliveira Rodini C, Batista AC, Lara VS. Comparative immunohistochemical study of the presence of mast cells in apical granulomas and periapical cysts: possible role of mast cells in the course of human periapical lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:59–63.
27. Juremalm M, Hjertson M, Olsson N, et al. The chemokine receptor CXCR4 is expressed within the mast cell lineage and its ligand stromal cell-derived factor-1alpha acts as a mast cell chemotaxin. Eur J Immunol 2000;30:3614–22.
28. Polajeva J, Sjosten AM, Lager N, et al. Mast cell accumulation in glioblastoma with a potential role for stem cell factor and chemokine CXCL12. PLoS One 2011;6:e25222.