Chemokine Monocyte Chemoattractant Protein-3 in Progressive Periodontal Lesions in Patients With Chronic Periodontitis

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Background: Chemokines are central in the activation and direction of leukocyte subsets to target tissues. However, the monocyte chemoattractant protein-3 (MCP-3) has not been associated with chronic periodontitis. Chronic periodontitis is an infection showing episodic supporting tissue destruction. The aim of this study is to determine the levels and expression of MCP-3 in periodontal sites characterized by active periodontal connective tissue destruction.

Methods: The study population consisted of 15 patients with a progression of periodontitis (15 of 56 patients), 18 patients with chronic periodontitis, and 10 healthy subjects without periodontal disease. As determined by the tolerance method, the 15 patients with moderate to advanced chronic periodontitis showed a progression of periodontitis over a 4-month period. Periodontitis was characterized by at least six sites with a probing depth ≥5 mm, clinical attachment level ≥3 mm, and radiographic bone loss. Gingival crevicular fluid was collected using a paper strip. The total protein concentration was determined. An enzyme-linked immunosorbent assay was performed to determine the total amount of MCP-3, and an immunoWestern blot was conducted to assess molecular MCP-3 forms. To determine the MCP-3 expression by immunohistochemistry, gingival biopsies were obtained from patients with chronic periodontitis and healthy subjects during third-molar extraction surgery. Statistical analyses were performed using statistical software. Data were expressed as subject means ± SD, using the χ^2 and Student t tests.

Results: The total amount and concentration of chemokine MCP-3 were significantly higher in patients with chronic periodontitis than in healthy subjects (8.25 pg versus 0.53 pg, $P = 0.006$ and 2.95 pg/µl versus 0.45 pg/µl, $P = 0.04$, respectively). Active sites showed a significantly higher total amount and concentration of MCP-3 than inactive sites (11.12 versus 2.88 pg, P value = 0.005 and 3.95 versus 1.02, P value = 0.005, respectively). Western blot and immunohistochemical staining confirmed the presence of MCP-3 in periodontal disease, with observable differences between patients with chronic periodontitis and healthy subjects.

Conclusions: MCP-3 was highly expressed in patients with chronic periodontitis, particularly in those with progressive periodontal lesions. MCP-3 could be involved in the recruitment of inflammatory cells toward periodontal tissues during the progression of the disease. *J Periodontol 2010;81:267-276.*

KEY WORDS

CCL7; chemokine; chronic periodontitis; cytokine; MCP-3; periodontitis.

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Chronic periodontitis is defined as an infectious disease leading to slow to moderate progressive loss of attachment and bone.[1] Disease initiation is due to bacterial infection, and there is evidence that only some bacterial species of biofilm adhering to the tooth surface contribute to the disease.[2] The clinical features of periodontitis include clinical attachment loss (AL), alveolar bone loss, periodontal pockets, and gingival inflammation.[3] There is abundant evidence that major tissue destruction in periodontal lesions results from the recruitment of host cells via the activation of monocytes/macrophages, lymphocytes, fibroblasts, and other cell types.[4,5] Considerable effort has been made to study the cytokines and chemokines released by different host cells when exposed to components of periodontopathogenic bacteria.[6] These studies demonstrated that a number of cytokines and chemokines are synthesized in response to periodontopathogenic bacteria, and their products induce and maintain an inflammatory response in the periodontium.[6,7]

Chemokines are a family of small (8 to 11 kDa) proteins that are capable of activating and promoting the vectorial migration of a variety of leukocytes, and they are classified into four groups according to the position of two highly conserved cysteine residues.[8] The monocyte chemoattractant proteins–1, –2, –3, and –4 (MCP-1/chemokine (C-C motif) ligand [CCL], MCP-2/CCL8, MCP-3/CCL7, and MCP-4/CCL13) constitute a small subfamily within the CC chemokine group; MCP-1 was the first to be identified, followed by MCP-2, -3, and -4.[9-13] MCP-1, an active chemoattractant of monocytes/macrophages, was detected in human gingival crevicular fluid (GCF) and inflammatory gingival tissue.[14,15] However, to our knowledge, MCP-3 has not been associated with chronic periodontitis. MCP-3 (also known as CCL7) was reported to interact with several C-C chemokine receptors, which can be simultaneously or selectively expressed in leukocyte subpopulations.[12] MCP-3 is expressed by endothelial cells and monocytes after induction by interleukin (IL)-1β, tumor necrosis factor-alpha (TNF-α), interferon (IFN)-γ, and lipopolysaccharide (LPS) and by fibroblasts and mononuclear cells after stimulation with IL-1β and IFN-γ.[13] The aims of the present study are: 1) to determine the total amount and concentration of MCP-3 in GCF; 2) to compare MCP-3 levels in GCF samples from active sites as determined by the tolerance method, which detected disease activity due to changes in clinical attachment levels; and 3) to assess the expression of MCP-3 in gingival tissue samples from patients with chronic periodontitis.

MATERIALS AND METHODS

Patients

This was a longitudinal clinical study in which patients with moderate to severe chronic periodontitis were followed until they developed periodontitis progression. Fifty-six patients were selected from the Center of Diagnosis and Treatment of Northern Metropolitan Health Service, Santiago, Chile, and consecutively enrolled between March 2005 and January 2006. The criteria for entry were a minimum of 14 natural teeth, excluding third molars and including at least 10 posterior teeth. Patients with chronic periodontitis had moderate to advanced periodontitis, where at least six teeth had sites with a probing depth (PD) ≥5 mm, clinical AL ≥3 mm, and extensive bone loss as assessed by radiography (>50% of support tissues involved, according to a classification system of periodontal disease severity[1] based on the location of the alveolar crest) and had received no periodontal treatment at the time of examination. The patients included in our study fulfilled the following criteria: no history of a systemic disorder, such as diabetes mellitus or osteoporosis; no medications known to influence periodontal tissues; no pregnant or lactating females; and no patients who had received antibiotic, anti-inflammatory, anticoagulant, or hormonal drugs within the 6-month period prior to the study. Before sampling, all patients received supragingival prophylaxis to remove gross calculus and to facilitate the determination of PD.

The protocol was clearly explained to all patients, and consent forms, which were approved by the Institutional Review Board and were the property of the Dental Faculty of the University of Chile, were signed. The protocol stated that, within 2 weeks of the detection of disease activity, including AL with pocketing, all patients would be provided with periodontal treatment. The research protocol stated that if a patient showed one or more teeth exhibiting AL in two consecutive or alternate evaluations, but at the same site, or if they presented with a periodontal abscess during the study period, the tooth or teeth would be treated and excluded from the study. The periodontal therapy consisted of scaling and root planing and oral hygiene instructions.

Clinical Measurements and Determination of Progressive Periodontitis

Clinical parameters were evaluated in all teeth, excluding third molars, and included PD, CAL, dichotomous measurements of supragingival plaque accumulation, and bleeding on probing (BOP) to the base of the crevice. Six sites were examined for each tooth: mesio-buccal, medio-buccal, disto-buccal, disto-lingual, medio-lingual, and mesio-lingual. Calibrated examiners (OR, JG) monitored the patients and collected the clinical reports.

Disease activity was defined by the tolerance method,[16] and clinical parameter measurements were monitored at baseline and 2 and 4 months. Sites were considered active if they exhibited AL ≥2.0 mm during a 2-month period. Inactive sites presented clinical AL, PD, and BOP that were equivalent to active sites (similar area, tooth, and face) but without AL during a 2-month period. A patient was considered to be undergoing disease progression when ≥2 active sites were diagnosed.

Fifteen patients (15 of 56 patients) showed progression of periodontitis during a 4-month period as determined by the tolerance method. Ten patients (10 of 56 patients; 17.85%) exhibited disease activity during the first 2 months, and five patients exhibited disease activity during the following 4-month period (five of 56 patients; 8.92%). A total of 15 patients underwent disease progression from the total of 56 patients with chronic periodontitis who were examined.

The study population consisted of 15 patients with a progression of periodontitis (15 of 56), 18 patients with chronic periodontitis, and 10 healthy subjects without periodontal disease. Samples of GCF and gingival biopsies were collected from all patients. One sample of GCF and one gingival biopsy were collected from each patient. In patients with periodontal disease progression, one active site and one inactive site from each patient undergoing AL were collected before the periodontal therapy.

Tissue-Sample Collection and Immunohistochemistry

From each patient, samples of gingival biopsy were collected from one active and one inactive periodontal lesion under troncular anesthesia and according to surgical therapy requirements. Gingival tissue biopsies were obtained from healthy subjects during third-molar extraction surgery in rehabilitation treatment. In patients with chronic periodontitis, one site with PD ≥5 mm and clinical AL ≥3 mm was taken. The incisions were made 1 to 2 mm subgingivally; therefore, specimens consisted of a gingival margin, sulcus epithelium, and gingival connective tissue. Tissue samples were fixed with formaldehyde and embedded in paraffin. The samples were deparaffinized and dehydrated, and the sections were blocked with normal serum followed by overnight incubation with an antibody against MCP-3.[¶] Endogenous peroxidase was blocked with H_2O_2, and the samples were incubated with a secondary antibody. Slides were stained with streptavidin-peroxidase, developed with 3,3-diaminobenzidine (DAB), and counterstained with hematoxylin. The same procedure was followed for negative controls but without incubating the first antibody.

GCF Collection and Elution

Samples were collected from 10 healthy subjects, 18 patients with chronic periodontitis, and 15 subjects undergoing a progression of periodontitis (15 active and 15 inactive sites). In patients with a progression of periodontitis, two sites (one active and one inactive site) were collected, and a biopsy was collected from one site in each chronic periodontitis and healthy subject. Sampled sites were isolated with cotton rolls and dried with a gentle stream of air to prevent saliva contamination. A sterile paper strip[#] was inserted into the gingival crevice and left in place for 30 seconds. After GCF collection, the volume of the sample at each paper strip was measured using a calibrated electronic gingival fluid measuring device.[**] The readings from the device were converted to an actual volume (microliters) by reference to the standard curve. The elution was performed with 100 µl elution buffer for 30 minutes at 4°C (Tris HCl, 50 mM; Triton X-100, 0.01%; and NaCl, 0.2 M) per strip, and centrifuged at 4°C and 12,500 rpm for 5 minutes. Aliquots of each eluate were saved for quantification of total proteins and enzyme-linked immunosorbent assays (ELISAs).

Quantifications of Total Protein Content

For quantification of the total protein content, a bicinchoninic acid[††] assay was used according to the manufacturer's instructions.

Western Blot Analysis

The expression of MCP-3 in GCF was assessed through an immunoWestern blot. We used samples of GCF from all patients included in this study, and each experiment was repeated three times. In patients with progression and with periodontitis, we used samples from sites with PD ≥5 mm and clinical AL ≥3 mm. Samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis Tris-glycine gels in non-reducing conditions and transferred to a polyvinylidene difluoride (PVDF) membrane. For Western hybridization, the membrane was blocked with 3% skim milk in Tris-buffered saline–Tween 0.1% (TBS-T) for 1 hour. The primary antibody against MCP-3,[‡‡] which was diluted 1:250 in 3% skim milk in TBS-T, was added, and the membrane was incubated overnight. The membrane was washed with TBS-T, incubated for 1 hour with horseradish peroxidase-conjugated secondary antibody, and washed again with TBS-T, and the hybridized bands were detected with an enhanced chemiluminescence detection kit.[§§]

Table 1.

Clinical Characteristics of Patients with Periodontitis, Controls, and Patients With a Progression of Periodontitis (mean ± SD)

Characteristic	Patients (n = 18)	Controls (n = 10)	Progression (n = 15)
Age (years; mean ± SD)	45.44 ± 6.50	44.34 ± 6.20	42.53 ± 4.43
Females (n)	10	6	9
PD (mm; mean ± SD)	3.98 ± 0.46*	1.20 ± 0.32*†	4.42 ± 0.46†
Attachment loss (mm; mean ± SD)	4.20 ± 0.52‡§	0.46 ± 0.22‡‖	6.20 ± 0.76§‖
Sites with plaque (%)	100¶	16.66¶	100
Sites with BOP (%)	100#	8.22#	100

* Mean PD in periodontitis versus control groups (unpaired t and χ^2 tests; $P = 0.001$).
† Mean PD in periodontitis progression versus control groups (unpaired t and χ^2 tests; $P = 0.0001$).
‡ Mean attachment loss in periodontitis versus control groups (unpaired t and χ^2 tests; $P = 0.0001$).
§ Mean attachment loss in periodontitis progression versus periodontitis groups (unpaired t and χ^2 tests; $P = 0.0001$).
‖ Mean attachment loss in periodontitis progression versus control groups (unpaired t and χ^2 tests; $P = 0.0001$).
¶ Percentage of sites with plaque in periodontitis versus control groups (unpaired t and χ^2 tests; $P = 0.001$).
# Percentage of sites with BOP in periodontitis versus control groups (unpaired t and χ^2 tests; $P = 0.0001$).

Concentration of MCP-3 by ELISA

The GCF level of MCP-3 was quantified using the sandwich ELISA assay‖ according to the manufacturer's instructions. The standards and samples, respectively, were incubated in a 96-well polystyrene microplate that was precoated with anti-MCP-3 antibody. The plate was read at 450 nm and corrected at 540 nm. The concentration of MCP-3 in GCF samples was determined by interpolation from a polynomial curve.

Statistical Analyses

Data analyses were performed using a statistical package.¶¶ Data comparisons of MCP-3 levels among the different conditions (healthy versus periodontitis and active sites versus inactive sites) were analyzed using the unpaired t test. Dichotomic measurements were analyzed by the χ^2 test. $P < 0.05$ was considered statistically significant.

RESULTS

The clinical characteristics of patients with chronic periodontitis and healthy volunteers included in this study are shown in Table 1. The periodontitis group consisted of eight males and 10 females (age range: 37 to 67 years; mean age: 45.44 ± 6.50 years), and the control group consisted of four males and six female (age range: 31 to 55 years; mean age: 44.34 ±

6.20 years). No statistically significant differences in age or gender existed between the two groups. In the group with chronic periodontitis, we observed significantly higher percentages of sites than the other two groups with plaque, BOP, reduced ALs, and deeper PDs ($P < 0.05$).

The clinical characteristics of 15 of the sites from patients with periodontitis undergoing progressive chronic periodontitis exhibited no statistical difference between active and inactive sites with regard to clinical parameters (Table 2). In both groups, 100% of the sites showed plaque and BOP.

Mean variations of total amounts per site and concentrations of MCP-3 in the periodontitis and control groups are shown in Table 3 and Fig. 1. Significantly higher levels were observed in the periodontitis groups compared to the control groups (8.25 ± 8.02 pg versus 0.53 ± 0.05 pg, $P = 0.006$, and 2.95 ± 2.80 pg/μl versus 0.45 ± 0.70 pg/μl, $P = 0.04$, respectively).

The mean variations of MCP-3 levels in patients with progressive chronic periodontitis are shown in Table 4 and Fig. 1. The total amount of MCP-3 per site was significantly higher in active sites than inactive sites (11.12 ± 8.04 versus 2.88 ± 2.41 pg; $P = 0.005$). When the cytokine concentration (picograms per microliter) was calculated from the volume of GCF estimated from the reading on the calibration unit, levels were also higher in active sites than inactive sites (3.95 ± 0.72 versus 1.02 ± 0.85; $P = 0.005$).

The immunohistochemistry of gingival tissue specimens from patients with chronic periodontitis revealed that MCP-3 was expressed in the gingival tissue from periodontitis, and it was localized to inflammatory cells, mainly plasmocytes and vascular endothelium; conversely, MCP-3 was not detected in healthy subjects (Fig. 2). MCP-3 immunoreactivities in GCF samples of patients with progressive periodontitis were seen at 13 kDa (Fig. 3). Fainter bands were observed in non-progressive lesions, and, again, no expression of MCP-3 was found in samples from healthy subjects. MCP-3 bands were observed in all samples from patients with periodontal disease. Healthy patients did not show any immunoreactivity.

DISCUSSION

Our results show, for the first time to our knowledge, the expression of MCP-3 in patients with chronic periodontitis. Through immunohistochemistry, we

Table 2.

Clinical Characteristics of Patients With Periodontitis and Active and Inactive Sites

Characteristic	Clinical Parameters	
	Active Sites (n = 15)	Inactive Sites (n = 15)
PD (mm; mean ± SD)	4.42 ± 0.46*	4.64 ± 0.65*
Attachment loss (mm; mean ± SD)	6.20 ± 0.76†	6.42 ± 0.68†

* *P* = 0.08.
† *P* = 0.16 (paired *t* test).

Table 3.

MCP-3 Levels in GCF From Patient (sites with PD ≥5 mm and Clinical AL ≥3 mm) and Control Groups

Characteristic	Patients (n = 18)	Controls (n = 10)	*P* Value (unpaired *t* test)
MCP-3 (pg; mean ± SD)*	8.25 ± 8.02	0.53 ± 0.05	0.006
MCP-3 (pg/μl; mean ± SD)†	2.95 ± 2.80	0.45 ± 0.70	0.04
GCF (μl; mean ± SD)	0.94 ± 0.20	0.22 ± 0.20	0.001
Total proteins (ug/μl; mean ± SD)	0.85 ± 0.16	0.17 ± 0.03	0.0001

* Total amount of MCP-3.
† Concentration of MCP-3.

demonstrated that gingival tissues from patients with chronic periodontitis expressed MCP-3, and we confirmed the presence of MCP-3 by an immunoWestern blot in GCF from those same patients. Our data demonstrates that chemokine MCP-3 was present in the GCF of adult patients with chronic periodontitis in a significantly higher amount than in healthy subjects, and the total amount of chemokine MCP-3 present in GCF of actives sites in patients with the progression of periodontitis was found in significantly higher levels than in the GCF of inactive sites. Considering the levels of MCP-3 found in the present study, it is possible to speculate that this cytokine is involved in the development of the gingival inflammatory response by mediating the activation of the immune response in chronic periodontitis and in the progression of periodontitis.

The expression of MCP-3 mRNA and protein secretion can be induced in response to a number of proinflammatory stimuli including IL-1β, TNF-α, LPS, and IFN-α, -β, and -γ,[17-20] and MCP-3 was implicated in the etiopathogenesis of several autoimmune and inflammatory diseases.[21-23] IL-1 is a proinflammatory cytokine that enables the recruitment of cells toward infection sites, promotes bone resorption, and stimulates prostaglandin release by monocytes and fibroblasts and the release of metalloproteinases that degrade extracellular matrix proteins.[24] The predominant form of IL-1 found in the periodontal tissues is IL-1β, which is primarily produced by macrophages.[25,26] Increased levels of IL-1 were reported in the inflammatory gingival tissue of patients with periodontitis,[27] IL-1 was detected in GCF from inflammatory periodontal tissues,[28] and elevated levels of IL-1 were associated with active sites and the progression of the disease.[29] IFN-γ, a cytokine with multiple immunoregulatory effects, mediates the host defense against infection and is a potent activator of mononuclear phagocytes.[30] The effect of T cells on osteoclastogenesis depends on the balance between the receptor activator of nuclear factor-kappa B ligand (RANKL) and IFN-γ.[31] In some studies,[32,33] IFN-γ seemed to be the predominant cytokine produced by T cells in periodontal diseases, and an enhancement of IFN-γ-producing cells was correlated with the progression of disease. All of this information demonstrates that MCP-3 could take part in the pathogenic events observed during the progression of chronic periodontitis and could partially explain the mechanisms associated with the destruction of the supporting tissues of the tooth. Our data shows the expression of MCP-3 in gingival tissues and GCF samples from patients with chronic periodontitis and suggests its role in leukocyte trafficking and, therefore, in the pathogenesis of periodontitis. The diversity of MCP-3 target cell types, as well as the capacity of MCP-3 to desensitize leukocyte responses to other C-C chemokines, suggests that MCP-3 may interact with multiple C-C chemokine receptors.[34] Furthermore, MCP-3 was mainly expressed in inflammatory leukocytes and the vascular endothelium, confirming its potential role in the recruitment of leukocytes to diseased gingival tissue.

As GCF traverses the inflamed tissue, it carries molecules involved in the destructive process and, therefore, represents a source of factors associated with osteoclastic activity, which could be detected in advance of irreversible bone loss. GCF has the benefit of being closely approximated to the site of destruction. Because periodontitis is regarded as
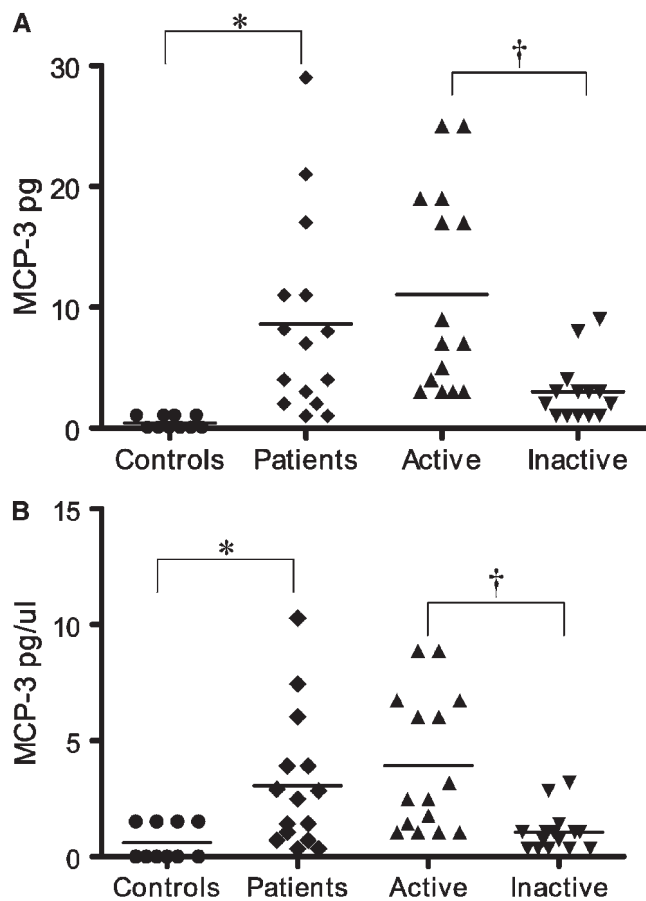
Figure 1.

*MCP-3 levels in the GCF from healthy controls, patients, and active and inactive sites. **A)** Total amount of MCP-3 per site in the control groups, chronic periodontitis groups, and patients undergoing progressive chronic periodontitis. **B)** Concentration of MCP-3 per site in the control groups, chronic periodontitis groups, and patients undergoing progressive chronic periodontitis. *P <0.05 (unpaired t test); †P <0.05 (paired t test).*

Table 4.

MCP-3 Levels in GCF From Active and Inactive Sites

Characteristic	Active (n = 15)	Inactive (n = 15)	P Value (paired t test)
MCP-3 (pg; mean ± SD)*	11.12 ± 8.04	2.88 ± 2.41	0.001
MCP-3 (pg/µl; mean ± SD)†	3.95 ± 0.72	1.02 ± 0.85	0.001

* Total amount of MCP-3.
† Concentration of MCP-3.

a site-specific disease, GCF can provide more information than markers in the serum or urine.[35,36] Several molecules were analyzed in GCF, including plasma proteins, enzymes with collagenolytic activity,[37] other microbial and host cell enzymes, and in-

flammatory mediators,[38,39] in attempts to identify factors to facilitate the diagnosis of active periodontal disease. In the present study, total amounts of MCP-3 in GCF from periodontitis subjects were higher than those in healthy subjects; this result was confirmed by the lack of MCP-3 detection by Western blot and immunohistochemical staining in the control samples. The prevalence of the expression of MCP-3 was detected in samples of healthy subjects when ELISA was used and was not detected when immunohistochemistry and the Western blot were used. These findings may suggest different sensitivities for antibody responses to the MCP-3 protein or a difference in the degree of inflammation. The specificity of the ELISA method is typically better, by orders of magnitude, than a Western blot due, in large part, to the fact that any protein must be bound simultaneously by both the capture antibody and the detection antibody. The immunohistochemical staining method may not be sensitive enough to detect chemokine and chemokine-receptor expression on infiltrating cells. Overall, these observations suggest that MCP-3 is implicated in the pathogenesis of chronic periodontitis.

Longitudinal studies[40,41] on the progression of periodontitis demonstrated that the rate of periodontal tissue destruction was rather low, whereas advanced forms of the disease occurred in comparatively few individuals and few tooth sites. Previously, attention was focused on the possibility that periodontal disease may not be a continuous process but could be characterized by episodes of activity, followed by periods of relative quiescence.[42] Episodic periodontal probing attachment loss could be associated with variations in supracrestal inflammatory cell populations, where significantly higher numbers of mast cells, monocytes/macrophages, and plasma cells are present in active sites compared to inactive sites.[4,43] Binding receptors for MCP-3 include CC chemokine receptor (CCR) 1, 2, 3, and 5; hence, the MCP-3 target population is mainly comprised of cells of the monocyte–macrophage lineage. However, the contribution of acquired immune cells in the progression of periodontal disease has long been controversial, with its exact role in the protection versus destruction of the host's periodontium remaining unclear.[44] One of the main concerns about chronic periodontitis is the definition of active sites, where tissue destruction and, probably, the secretion of certain cytokines are occurring. Previously, we showed higher levels of CD4+ helper T cells in the context of periodontal disease progression, which could directly trigger osteoclastogenesis and alveolar bone loss associated with periodontitis in vivo.[45] MCP-3 is a powerful chemotactic protein that elicits the infiltration of monocytes, activated T lymphocytes, eosinophils, and
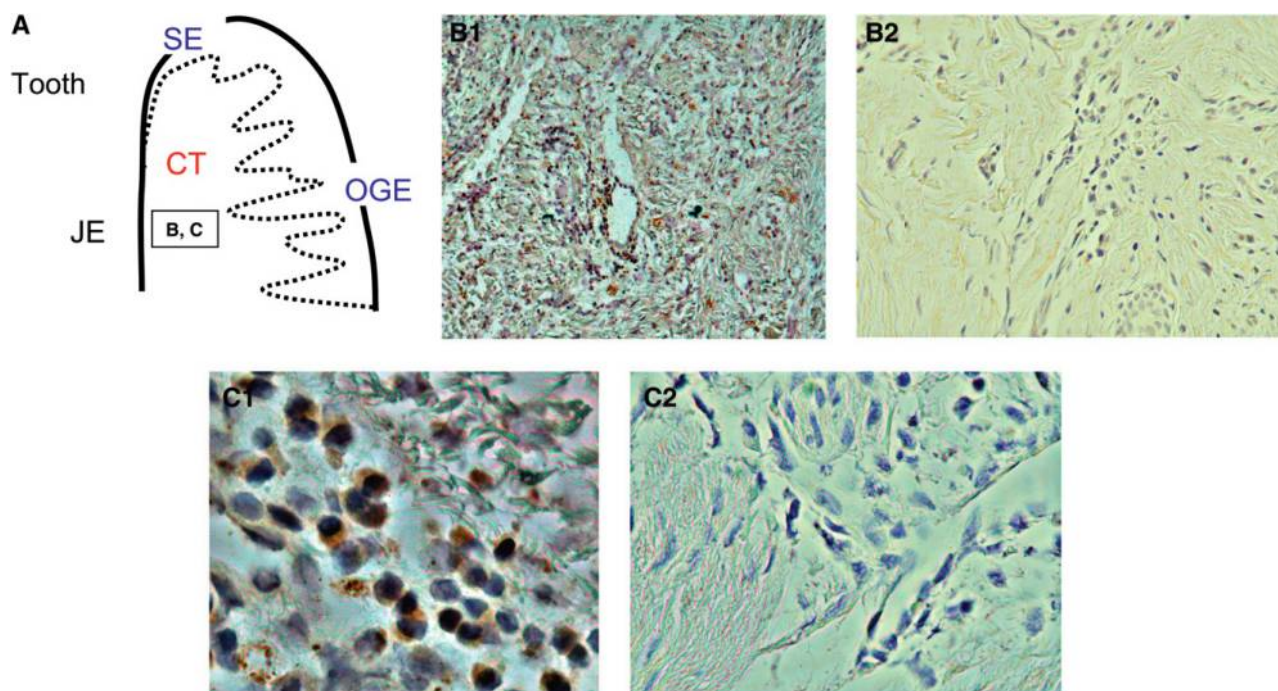
Figure 2.
*MCP-3 immunohistochemistry in gingival tissue from patients with chronic periodontitis and healthy subjects. **A)** Diagram illustrating the localization of sections in panels **B through C**. B1 and C1) Gingival biopsy from healthy subjects. B2 and C2) Gingival biopsy from patients with chronic periodontitis stained with anti-human MCP-3. Specimens were immunostained with anti-human MCP-3 using a DAB chromogen and hematoxylin counterstain. JE = junctional epithelium; SE = sulcular epithelium; CT = infiltrated connective tissue; OGE = orogingival epithelium. (Original magnification: B, ×10; C, ×40).*
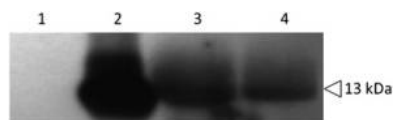

Figure 3.
MCP-3 immunoreactivities in the GCF of patients with chronic periodontitis. ImmunoWestern blot showing representative samples from each study group: lane 1 = healthy; lane 2 = positive control recombinant MCP-3; lane 3 = active sites; and lane 4 = inactive sites. GCF aliquots were run in non-reducing conditions in Tris-glycine gels, transferred to the PVDF membrane, incubated with MCP-3 antibody, and visualized by the chemiluminescence method. Immunoreactivities of 13 kDa are seen in all samples except healthy GCF.

basophils.[46,47] Our findings demonstrate that the level of MCP-3 was significantly higher in actives sites versus inactive sites ($P < 0.05$) and could be considered a marker of progression. However, samples of periodontitis showed higher levels of MCP-3 than inactive sites from patients with a progression of periodontitis (8.25 pg versus 2.88 pg), and inactive sites of periodontitis progression demonstrated that the rate of periodontal tissue destruction was rather low, whereas advanced forms of the disease occurred in comparatively few individuals and few tooth sites. Consistently, our study showed that patients with chronic periodontitis had increasing MCP-3 levels, and these observations suggest that the augmented expression of MCP-3 abnormally recruits inflammatory cells into the periodontal tissue, mediating the initiation and progression of chronic periodontitis.

Although mechanisms controlling osteoclast development from hematopoietic precursors and the essential role of the RANKL/RANK/osteoprotegerin system were well described,[48,49] relatively little is known about specific pathways that govern the recruitment of circulating preosteoclasts into the bone marrow and direct them to appropriate stromal niches and bone remodeling sites for their development and function in normal or pathologic states. A recent study[50] showed that murine RAW 264.7 macrophage cells and primary bone-marrow preosteoclast populations expressed and upregulated the chemokine receptor CCR1 during their RANKL-induced osteoclast formation. CCR1-binding ligands (macrophage inflammatory protein 1 alpha [MIP1α], RANTES, and MCP-3) further stimulate the chemoattraction and RANKL-dependent development of preosteoclast and the motility of mature osteoclasts. Furthermore, these chemokines are produced in a development- or inflammatory-dependent manner

by osteoblasts, osteoclasts, or their precursors.[50] MIP1α, RANTES, and MCP-3 chemoattract various leukocyte subsets are produced by multiple cell types, increase in response to inflammatory stimuli, and are important in recruiting and activating cells in osteolytic diseases like rheumatoid arthritis.[51] Our previous data demonstrated that higher RANKL levels in patients with periodontitis were associated with gingival CD4+ T cells during periodontal infection.[52]

The attraction of leukocytes to sites of inflammation and infection is an essential component of the host response to disease. Chemokines and chemokine receptors are an integral part of this process and have been implicated in the pathophysiology of many infectious and inflammatory diseases.[8,34] Although chemokines are clearly important for the ability of the host to control infections, they can also be detrimental in certain inflammatory diseases where inflammatory cells are recruited into tissue sites causing an inflammatory infiltrate, which results in tissue damage. In such disorders, it has been suggested that chemokines and their receptors could be used as therapeutic targets for controlling pathologic inflammation.[53] Taking these data together, we propose that tissue destruction adjacent to the inflamed area in periodontitis might be regulated by MCP-3 activity through the trafficking, localization, and activation of various hematopoietic cell types. Nevertheless, to our knowledge, no studies investigated the MCP-3 concentrations in the periodontia of patients with chronic periodontitis alone and compared them to the MCP-3 levels in patients with progressive periodontitis and healthy subjects. Moreover, a possible correlation between the MCP-3 concentration and diseased nature of the periodontium has not been revealed. To our knowledge, this is the first study that attempted to reveal data about these points.

CONCLUSION

This study provides new and interesting findings regarding the levels of MCP-3 in patients with chronic periodontitis and in patients with an episodic loss of connective tissue, suggesting that MCP-3 has a potential role as a mechanism of tissue destruction associated with destructive periodontitis.

ACKNOWLEDGMENTS

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