Journal of
PERIODONTAL RESEARCH

J Periodont Res 2016; 51: 471–480

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/jre.12324

Gingival crevicular fluid interleukin-8 and lipoxin A_4 levels of smokers and nonsmokers with different periodontal status: a cross-sectional study

M. Lütfioğlu[1], A. Aydoğdu[2], E. E. Sakallioğlu[1], H. Alaçam[3], F. Pamuk[4]

[1]Department of Periodontology, OndokuzMayis University Faculty of Dentistry, Samsun, Turkey, [2]Department of Periodontology, Biruni University Faculty of Dentistry, İstanbul, Turkey, [3]Department of Medical Biochemistry, Hacettepe University Medical Faculty, Ankara, Turkey and [4]Department of Periodontology, Istanbul Aydin University Faculty of Dentistry, İstanbul, Turkey

Lütfioğlu M, Aydoğdu A, Sakallioğlu EE, Alaçam H, Pamuk F. Gingival crevicular fluid interleukin-8 and lipoxin A_4 levels of smokers and nonsmokers with different periodontal status: a cross-sectional study. J Periodont Res 2016; 51: 471–480.

Background and Objective: Smoking is an important risk factor for periodontal disease and effects the pathogenesis of the disease. This study evaluated the impact of smoking on gingival crevicular fluid interleukin-8 (IL-8) and lipoxin A_4 (LxA_4) levels in patients with and without periodontal disease.

Material and Methods: A total of 122 participants were grouped as follows: smokers with generalized aggressive periodontitis (S-GAgP, $n = 15$); smokers with chronic periodontitis (S-CP, $n = 17$); smokers with gingivitis (SG, $n = 15$); smokers classified as periodontally healthy (SH, $n = 15$); nonsmokers with generalized aggressive periodontitis (N-GAgP, $n = 15$); nonsmokers with chronic periodontitis (N-CP, $n = 15$); nonsmokers with gingivitis (NG, $n = 15$); and nonsmokers classified as periodontally healthy (NH, $n = 15$). Gingival index, plaque index, probing pocket depth and clinical attachment level were recorded. Gingival crevicular fluid IL-8 and LxA4 levels were analyzed by ELISA.

Results: Gingival crevicular fluid IL-8 levels varied among groups, as follows: S-GAgP>S-CP>SG>SH and N-GAgP>N-CP>NG>NH. The gingival crevicular fluid IL-8 levels were significantly higher in the S-GAgP group compared with the N-GAgP group and in the S-CP group compared with the N-CP group ($p < 0.05$); differences between the SG and NG and the SH and NH groups were not statistically significant ($p > 0.05$). Gingival crevicular fluid LxA_4 levels also varied among groups, but in an inverse direction when compared with the IL-8 levels, as follows: S-GAgP<S-CP<SG and N-GAgP<N-CP<NG. (The gingival crevicular fluid LxA_4 levels in SH and NH groups were below the limits of detection.) The gingival crevicular fluid LxA_4 levels were significantly lower in the S-GAgP group than in the N-GAgP group and in the S-CP group than in the N-CP group ($p < 0.05$); differences between the SG and NG groups were not statistically significant ($p > 0.05$).

Conclusion: The study findings suggest that the observed increases in gingival crevicular fluid IL-8 levels and decreases in gingival crevicular fluid LxA_4 levels

Müge Lütfioğlu, PhD, DMD, Department of Periodontology, Faculty of Dentistry, University of Ondokuz Mayis 55139, Kurupelit, Samsun, Turkey
Tel: +903623121919 2155
Fax: + 903624576032
e-mail: mugelutfioglu@hotmail.com

Key words: inflammatory mediator; inflammation; periodontal disease; smoking; interleukin 8; lipoxin A4

Accepted for publication August 13, 2015

reflect changes in immune and inflammatory responses that occur as a result of smoking.

Periodontitis is a leukocyte-mediated inflammatory disease characterized by inflammation of the supporting tissues of the teeth induced by microorganisms that stimulate host immune and inflammatory responses (1). Polymorphonuclear leukocytes (PMNs) are the main type of phagocytic cells that protect periodontal tissue against bacterial infection and invasion; however, if not controlled, PMN infiltration at sites of infection may culminate in host-tissue damage (1,2). To ensure healthy tissue status, the initiation of acute inflammation and its resolution must remain efficient (3). As one of the principal mediators of the inflammatory response, interleukin-8 (IL-8) is an important chemoattractant of PMNs (4), making down-regulation and/or control of IL-8 production vital in preventing chronic inflammation and tissue destruction (5).

Until recently, the loss of pro-inflammatory mediators was widely accepted as a passive event in which the inflammation signal was 'turned off', putting an end to any further response (3,6). However, the resolution of inflammation and return to homeostasis has now come to be recognized as a highly regulated, biochemical process (3) – a 'programmed resolution' at the tissue level (3,7–12) – actively driven by specialized lipid immune resolvents that include resolvins, lipoxins, protectins and maresins (3,7–9,12). Lipoxins (LxA_4 and LxB_4) are a group of lipoxygenase-derived biologically active eicosanoids produced by PMNs, platelets, eosinophils and monocytes/macrophages (6). Lipoxin A_4 (LxA_4) limits PMN migration to the site of inflammation, promotes PMN apoptosis and activates the transformation of monocytes/macrophages from a phlogistic (i.e. heat- or fever-producing) phenotype to a nonphlogistic phenotype (7) that efficiently removes apoptotic PMNs and enhances clearance of the inflammation site (3,6–8). If inflammatory cell apoptosis is delayed and apoptotic inflammatory cells and their noxious products are not removed, chronic, pathological lesions will develop as a result (6,12–14). PMN priming and/or hyperfunction can also cause dysregulated chemotaxis, phagocytic abnormalities and heightened pro-inflammatory activity, such as increased oxidative stress and secretion of inflammatory mediators, which may lead to excessive tissue damage rather than killing of bacteria (7,13,14).

PMN-mediated destruction and inflammation are major causes of human inflammatory pathologies, including arthritis, asthma, cancer, cardiovascular disease and periodontal disease. Loss of resolution and failure to return tissue to homeostasis results in PMN-mediated destruction and chronic inflammation (3). However, the role of novel lipid mediators in the pathogenesis of periodontal disease is still unclear. Given that studies have implicated lipid mediators in the prevention of inflammation by limiting PMN recruitment, Lx and other mediators have been targeted by new research into possible strategies for controlling the extent and severity of periodontal disease (7,9,13,15,16).

Cigarette smoking is also known to be a major risk factor for periodontal disease and to affect the disease's pathogenesis (17). Smoking alters the host response, including vascular function, PMN/monocyte activity, adhesion molecule expression, antibody production and the release of cytokines and inflammatory mediators (18–21). The mechanisms connecting smoking and periodontal disease are believed to include the relative inhibition and/or alteration of the normal host immune and inflammatory responses, particularly in terms of PMN function (20,22–24).

Therefore, based on our understanding that IL-8 and LxA_4 play important roles in determining neutrophil function in the inflammatory response described above, the objective of the present study was to evaluate the influence of cigarette smoking on neutrophil-related mediators (IL-8 and LxA_4) in gingival crevicular fluid of periodontally healthy individuals and of those with varying degrees of periodontal disease.

Material and methods

Study population

This cross-sectional study was conducted with 122 patients (60 male and 62 female subjects) recruited from the Periodontology Department at the Ondokuz Mayis University Faculty of Dentistry in Samsun, Turkey, between September 2012 and March 2014. The participants were randomly selected from individuals referred to the Periodontology Department either for dental treatment or only for a dental check-up. The study protocol was approved by the Local Ethics Committee, and written informed consent was obtained from all study participants, in accordance with the Helsinki Declaration (ISRCTN registration no: ISRCTN13030013).

Inclusion criteria were as follows: (i) ≥18 years of age and having ≥ 16 teeth; (ii) no periodontal therapy in the 6 mo before data collection; and (iii) no systemic problems or chemotherapy within the 6 wk before data collection. Individuals were included as smokers if they had smoked ≥15 cigarettes/d for ≥5 years, whereas individuals were included as nonsmokers if they had no previous history of smoking. Exclusion criteria were as follows: (i) medical history of cancer, rheumatoid arthritis, diabetes mellitus or cardiovascular disease; (ii) compromised immune system; (iii) pregnancy, menopause or lactation; (iv) ongoing drug therapy that might affect the clinical characteristics of periodontitis; (v) use of systemic

antimicrobials in the 6 wk before data collection; and (vi) dental treatment in the 6 mo before data collection.

Periodontal status was assessed by clinical examination, with classifications given according to criteria proposed by the 1999 International World Workshop for a Classification of Periodontal Disease and Conditions (25). A diagnosis of generalized chronic periodontitis was based on radiographic evidence of alveolar bone and attachment loss, with probing pocket depths of ≥5 mm for at least eight teeth. A diagnosis of generalized aggressive periodontitis was based on evidence of severe alveolar bone and attachment loss, with probing pocket depths of ≥5 mm for at least eight teeth, including three teeth that were not first molars or incisors. This diagnosis was supported by other factors, namely family aggregation, rapid progression and the lack of correlation between periodontal destruction and the local presence of calculus and dental plaque. Rapid progression was determined according to clinical, radiographic and overall etiologic manifestations of aggressive periodontitis.

Accordingly, study participants were grouped as follows: smokers with generalized aggressive periodontitis (S-GAgP); smokers with chronic periodontitis (S-CP); smokers with gingivitis (SG); periodontally healthy smokers (SH); nonsmokers with generalized aggressive periodontitis (N-GAgP); nonsmokers with chronic periodontitis (N-CP); nonsmokers with gingivitis (NG); and periodontally healthy nonsmokers (NH).

All clinical examinations and gingival crevicular fluid collection were performed by a single examiner. All laboratory procedures were performed by another researcher blinded to the study.

Clinical assessment

The following clinical parameters were recorded: Silness & Löe plaque index (PI) (26); Löe & Silness gingival index (GI) (27); probing pocket depth; clinical attachment level; and bleeding on probing (BOP). Measurements were performed on six sites per tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual and distolingual locations) using a Williams periodontal probe (Nordent Manufacturing Inc., Elk Grove Village, IL, USA), calibrated in mm. The deepest six sites of each subject were chosen for clinical recordings and further gingival crevicular fluid sampling.

Gingival crevicular fluid sampling and processing

A total of 102 gingival crevicular fluid samples were taken from the S-CP group (17 subjects × six sites) and 90 were taken from each of the remaining seven groups (15 subjects per group × six sites) using the sites with the greatest probing pocket depth for each patient.

Gingival crevicular fluid samples were collected using periopaper strips (Oraflow Inc., Plainview, NY, USA). Before sample collection, each site was gently air-dried, all supragingival plaque was removed and the area was carefully isolated to prevent samples from being contaminated with saliva. The paper strip was inserted into the gingival crevice up to 1 mm, or until mild resistance was felt, and was left in place for 30 s. Care was taken to avoid mechanical injury of gingival tissue, and any strip contaminated by bleeding or exudate was discarded. Gingival crevicular fluid sample volume (μL) was measured using a calibrated Periotron 8000 (Periotron® 8000; Pro Flow Inc., Amityville, NY, USA). Strips were individually placed in 500-μL plastic Eppendorf microcentrifuge tubes that were labeled, sealed with paraffin and stored at −70°C until required for processing (28).

Gingival crevicular fluid ELISA analysis for IL-8 and LxA_4

Gingival crevicular fluid elution was performed according to Curtis *et al.* (29), with slight modification. A total of 150 μL of 2% bovine serum albumin (0.01 M, pH 7.2) in phosphate-buffered saline was added to each eppendorf tube, and the samples were incubated at 4°C for 60 min. Following incubation, a sterile drill was used to bore a hole in the bottom of each tube, which was then placed inside a 1.5-mL tube, and the nested tubes were centrifuged at 10,000 *g* for 10 min at 4°C. This process was repeated twice in order to obtain 300 μL of gingival crevicular fluid eluate.

IL-8 (catalogue number: KAP1301; DIAsource ImmunoAssays SA, Louvain-la-Neuve, Belgium) and LxA_4 (catalogue number: CSB-E09689 h; Cusabio Biotech. Co. Ltd., Wuhan, Hubei Province, China) levels in gingival crevicular fluid samples were evaluated using standard ELISAs, according to the manufacturer's instructions. Enzyme–substrate reactions were terminated by the addition of an acid solution, and color change was measured spectrophotometrically at a wavelength of 450 nm. IL-8 and LxA_4 concentrations were identified using standard curves. After measuring the amounts of IL-8 and LxA_4 collected from each sample in a 30-s period, the concentrations of IL-8 and LxA_4 per sample (pg/μL) were calculated by multiplying the results of ELISA assays by a dilution coefficient [phosphate-buffered saline volume (μL) + gingival crevicular fluid volume (μL)/phosphate-buffered saline volume (μL)]. The total amounts of IL-8 and LxA_4 per sample (pg) were calculated by multiplying the concentrations per sample (pg/μL) with the gingival crevicular fluid volume. Units (μL) were converted and recorded in mL to simplify statistical analysis.

Statistical analysis

Statistical analysis was performed using the statistical software program SPSS (SPSS v. 21.0 Inc., Chicago, IL, USA), and the results are presented as mean and standard error of mean for numeric variables and as median and 25^{th}–75^{th} percentiles for ordinal variables (PI and GI). A Shapiro–Wilk test performed before parametric analysis showed normal distribution of data, and Levene's test showed nonhomogeneity of variance; therefore, Welch's ANOVA was used to

identify statistical differences among the groups, and Tamhane's tests were used for post-hoc group comparisons of the numeric variables. Comparisons among the ordinal variables of the study groups were performed using the Kruskal–Wallis test and, when there were significant differences ($p < 0.05$), post-hoc two-group comparisons were assessed using the Bonferroni-corrected Mann–Whitney *U*-test, and $p < 0.008$ was considered to be statistically significant. Pearson's correlation tests were used to identify relationships between clinical indices and gingival crevicular fluid IL-8 and LxA_4 levels. $p < 0.05$ was considered statistically significant. The primary outcome variable, gingival crevicular fluid IL-8 level, was used to decide the sample size calculation and determine the power of the study. Power analysis calculations indicated a minimum requirement of 15 participants per group in order to compare data between smokers and nonsmokers at $\alpha = 0.05$ with a power value of 80%.

Results

The distributions of gender and age of the groups were as follows: S-GAgP (seven male and eight female subjects; mean age: 30.40 ± 1.65 years); S-CP (nine male and eight female subjects; mean age: 42.17 ± 1.07 years); SG (eight male and seven female subjects; mean age: 28.20 ± 1.70 years); SH (eight male and seven female subjects; mean age: 27.73 ± 1.29 years); N-GAgP (seven male and eight female subjects; mean age: 32.11 ± 1.40 years); N-CP (seven male and eight female subjects; mean age: 45.13 ± 1.85 years); NG (eight male and seven female subjects; mean age: 25.06 ± 0.99 years); and NH (nine male and six female subjects; mean age: 29.2 ± 1.95 years). No significant differences were found between the study groups in terms of gender ($p > 0.05$) or age, with the exception of the age of the S-CP group in comparison with that of the S-GAgP, SG and SH groups, and the age of the N-CP group in comparison with that age of the N-GAgP, NG and NH groups ($p < 0.001$).

Clinical findings

The findings for the clinical parameters are presented in Table 1. For both smokers and nonsmokers, PI, GI and BOP scores and gingival crevicular fluid volumes were significantly higher in all groups with periodontal disease (S-CP, S-GAgP, SG, N-CP, N-GAgP and NG) when compared with the healthy groups (SH and NH) ($p < 0.05$). BOP scores and gingival crevicular fluid volumes were also significantly higher for the S-GAgP and S-CP groups when compared with the SG group and for the N-GAgP and N-CP groups when compared with the NG group ($p < 0.001$). Mean probing pocket depth and clinical attachment level scores were significantly higher for the S-GAgP and S-CP groups in comparison with the SG and SH groups ($p < 0.001$), but did not vary significantly between the S-GAgP and S-CP groups ($p > 0.05$). Similarly, mean probing pocket depth and clinical attachment level scores were significantly higher for the N-GAgP and N-CP groups in comparison with the SG and SH groups ($p < 0.001$), but did not vary significantly between the N-GAgP and N-CP groups, the SG and SH groups, or the NG and NH groups ($p > 0.05$).

No differences in any of the clinical parameters were found between smokers and nonsmokers with the same periodontal health status ($p > 0.05$).

Gingival crevicular fluid analysis

Cytokine levels in gingival crevicular fluid at the sampling sites are shown

Table 1. Clinical parameters of the sampling sites in the study groups

Study group	PI	GI	Probing pocket depth (mm)	Clinical attachment level (mm)	BOP (%)	Gingival crevicular fluid (μL)
S-GAgP	2.27 (1.56–2.87)‡	2.00 (1.40–2.36)‡	7.08 ± 0.21*	7.44 ± 0.23*	83.70 ± 3.81*	0.63 ± 0.05*
S-CP	2.36 (1.75–2.77)‡	1.80 (1.44–2.00)‡	5.69 ± 0.74*	6.67 ± 0.17*	72.57 ± 3.94*	0.64 ± 0.04*
SG	1.80 (1.00–2.80)‡	1.55 (1.16–2.00)‡	2.14 ± 0.15#	2.14 ± 0.15#	12.37 ± 3.97#	0.31 ± 0.01#
SH	0.38 (0.00–1.00)	0.00 (0.00–0.00)	1.40 ± 0.11	1.40 ± 0.11	1.42 ± 0.36	0.20 ± 0.01
N-GAgP	2.72 (2.05–2.97)‡‡	2.60 (2.00–2.86)‡‡	7.07 ± 0.36**	7.51 ± 0.33**	70.36 ± 4.25**	0.72 ± 0.03**
N-CP	2.44 (1.50–2.91)‡‡	2.00 (1.60–2.60)‡‡	6.13 ± 0.15**	6.92 ± 0.20**	85.36 ± 2.79**	0.69 ± 0.04**
NG	2.40 (2.00–2.60)‡‡	2.00 (1.25–2.05)‡‡	2.26 ± 0.14##	2.26 ± 0.14##	11.65 ± 3.12##	0.35 ± 0.03##
NH	0.20 (0.00–1.00)	0.00 (0.00–0.30)	1.41 ± 0.08	1.41 ± 0.08	1.24 ± 0.36	0.20 ± 0.01

Values are given as mean ± standard error of the mean for numeric variables and as median (25^{th} –75^{th} percentile) for ordinal variables.
*Significantly different from SG and SH groups (Welch's ANOVA, $p < 0.001$; post-hoc Tamhane test, $p < 0.001$).
#Significantly different from the SH group (Welch's ANOVA, $p < 0.001$; post-hoc Tamhane test, $p < 0.05$).
‡Significantly different from the SH group (Kruskal–Wallis test, $p < 0.001$; Mann–Whitney *U*-test with Bonferroni Correction, $p < 0.008$).
**Significantly different from NG and NH groups (Welch's ANOVA, $p < 0.001$; post-hoc Tamhane test, $p < 0.001$).
##Significantly different from the NH group (Welch's ANOVA, $p < 0.0001$; post-hoc Tamhane test, $p < 0.05$).
‡‡Significantly different from the NH group (Kruskal–Wallis test, $p < 0.001$; Mann–Whitney *U*-test with Bonferroni correction, $p < 0.008$).
BOP, bleeding on probing; GI, gingival index; N-CP, nonsmokers with chronic periodontitis; NG, nonsmokers with gingivitis; N-GAgP, nonsmokers with generalized aggressive periodontitis; NH, periodontally healthy nonsmokers; PI, plaque index; S-CP, smokers with chronic periodontitis; SG, smokers with gingivitis; S-GAgP, smokers with generalized aggressive periodontitis; SH, periodontally healthy smokers.

in Fig. 1A and B and in Table 2. In general, gingival crevicular fluid IL-8 levels increased with increases in periodontal destruction. For both smokers and nonsmokers, the concentrations and total amounts of gingival crevicular fluid IL-8 were significantly higher in all the groups with periodontal disease (S-GAgP, S-CP, SG, N-GAgP, N-CP and NG) compared with the healthy groups (SH and NH) ($p < 0.05$). The concentrations and total amounts of IL-8 in gingival crevicular fluid were also significantly higher in the S-GAgP group compared with the SG group; in the N-GAgP group compared with the NG group; in the S-CP group compared with the SG group; and in the N-CP group compared with the NG group ($p < 0.05$) (Table 2). The concentrations and total amounts of gingival crevicular fluid IL-8 were higher in the S-GAgP group compared with the S-CP group and in the N-GAgP group compared with the N-CP group, but these differences were not statistically significant ($p > 0.05$). Moreover, the concentrations and total amounts of gingival crevicular fluid IL-8 were significantly higher in the S-GAgP group compared with the N-GAgP group and in the S-CP group compared with the N-CP group ($p < 0.05$) (Fig. 1A and B). Finally, the concentrations and total amounts of gingival crevicular fluid IL-8 were higher in the SG group than the NG group and in the SH group than the NH group, but these differences were not statistically significant ($p > 0.05$) (Fig. 1A and B).

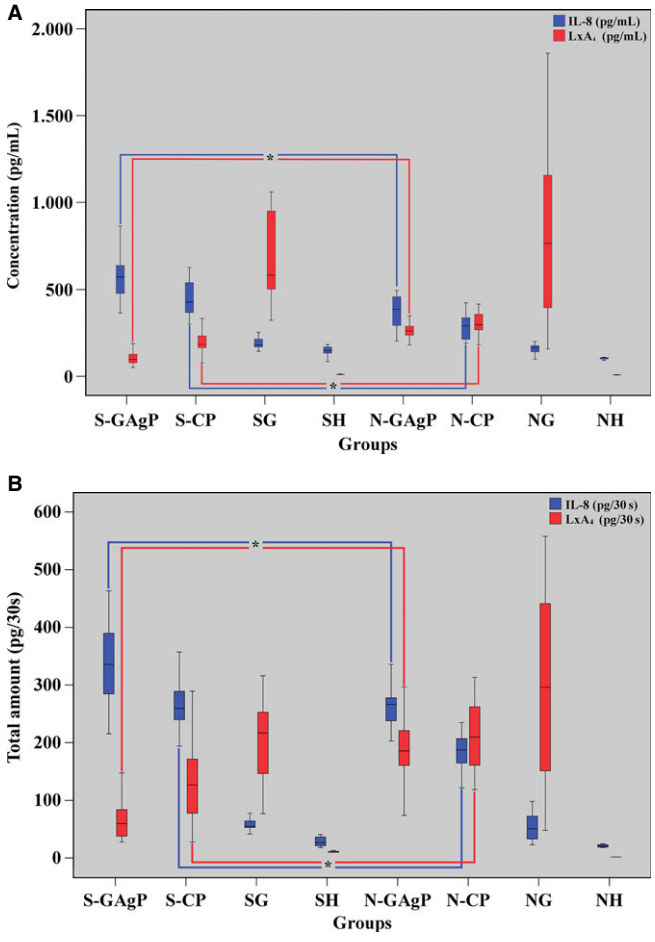

Fig. 1. Concentrations (A) and total amounts (B) of interleukin-8 (IL-8) and lipoxin A4 (LxA_4) in gingival crevicular fluid of the sampling sites. *Statistically significant difference between the groups (Welch's ANOVA $p < 0.001$; post-hoc Tamhane test, $p < 0.05$). N-CP, nonsmokers with chronic periodontitis; NG, nonsmokers with gingivitis; N-GAgP, nonsmokers with generalized aggressive periodontitis; NH, periodontally healthy nonsmokers; S-CP, smokers with chronic periodontitis; SG, smokers with gingivitis; S-GAgP, smokers with generalized aggressive periodontitis; SH, periodontally healthy smokers.

In contrast to gingival crevicular fluid IL-8 levels, the level of gingival crevicular fluid LxA_4 tended to decrease with increases in periodontal destruction. The gingival crevicular fluid LxA_4 levels in SH and NH groups were below the limits of detection by ELISA. For both smokers and nonsmokers, gingival crevicular fluid LxA_4 concentrations were significantly lower in the periodontitis groups than in the gingivitis groups ($p < 0.01$). The gingival crevicular fluid LxA_4 concentrations were also significantly lower in the S-GAgP group than in the S-CP group ($p < 0.05$). The gingival crevicular fluid LxA_4 concentrations were lower in the N-GAgP group than in the N-CP group, but this difference was not statistically significant ($p > 0.05$). The total amounts of gingival crevicular fluid LxA_4 were also lower in the periodontitis groups than in the gingivitis groups for both smokers and nonsmokers, but the difference was only statistically significant for the smokers ($p < 0.05$). The total amounts of gingival crevicular fluid LxA_4 were also lower in the S-GAgP group than in the S-CP group and in the N-GAgP group than in the N-CP group, but only the difference between the S-GAgP and SCP groups was statistically significant ($p < 0.05$). The gingival crevicular fluid LxA_4 levels were significantly lower in the S-GAgP group than in the N-GAgP group ($p < 0.001$) and in the S-CP group than in the N-CP group ($p < 0.05$) (Fig. 1A and B). The gingival crevicular fluid LxA_4 levels were lower in the SG group than in the NG group, but the difference was not statistically significant ($p > 0.05$) (Fig. 1A and B).

Correlations between cytokine levels and clinical periodontal parameters

For both smokers and nonsmokers, PI, GI, probing pocket depth, clinical

attachment level and BOP values were found to correlate positively with gingival crevicular fluid IL-8 levels and negatively with gingival crevicular fluid LxA_4 levels (Table 3). In addition, a negative correlation was found between gingival crevicular fluid IL-8 levels and gingival crevicular fluid LxA_4 levels (Table 4).

Discussion

PMNs play an essential role in host defence. However, despite their undeniably crucial role in the prevention of inflammatory injuries, their prolonged activation can lead to tissue injury and deleterious sequelae. Accordingly, although sufficient amounts of IL-8 are required to attract PMNs to a site of inflammation, excessive amounts can lead to PMN-mediated local tissue destruction (30).

The present study found that gingival crevicular fluid IL-8 levels in general increased with increases in the periodontal destruction for both smokers and nonsmokers. IL-8 values were slightly higher among both smokers and nonsmokers with generalized aggressive periodontitis than among those with chronic periodontitis, although the differences were not statistically significant. These findings are in line with those of Liu *et al.* (31), who reported that the total amounts of IL-8 in gingival crevicular fluid did not vary significantly between patients with chronic periodontitis and those with generalized aggressive periodontitis. Rescala *et al.*

Table 2. Concentrations and total amounts of gingival crevicular fluid interleukin-8 (IL-8) and lipoxin A_4 (LxA_4) in the study groups

Studygroup	IL-8 (pg/mL) concentration	IL-8 (pg/30 s) total amount	LxA_4 (pg/mL) concentration	LxA_4 (pg/30 s) total amount
S-GAgP	566.53 ± 37.27*,‡	338.98 ± 19.14*,‡	106.65 ± 10.99‡, §,‡	67.75 ± 9.13‡, §,‡
S-CP	443.37 ± 22.36*,‡	269.04 ± 10.77*,‡	202.08 ± 18.28‡,‡	135.09 ± 18.13‡,‡
SG	192.88 ± 11.00#	58.64 ± 3.02#	682.47 ± 64.78	207.96 ± 18.53
SH	144.63 ± 7.38	28.60 ± 2.18	–	–
N-GAgP	375.05 ± 25.88**,‡	258.04 ± 10.64**,‡	257.48 ± 14.77‡‡,‡	186.21 ± 14.89‡
N-CP	284.98 ± 21.28**,‡	185.90 ± 8.22**,‡	317.94 ± 20.92‡‡,‡	214.93 ± 15.91‡
NG	159.72 ± 10.24##	50.77 ± 6.41##	871.16 ± 133.68	285.06 ± 43.82
NH	106.57 ± 7.36	20.33 ± 0.58	–	–

Values are given as mean ± standard error of the mean.
*Significantly different from SG and SH groups (Welch's ANOVA, $p < 0.001$; post-hoc Tamhane test, $p < 0.001$).
#Significantly different from the SH group (Welch's ANOVA, $p < 0.001$; post-hoc Tamhane test, $p < 0.05$).
**Significantly different from NG and NH groups (Welch's ANOVA, $p < 0.001$; post-hoc Tamhane test, $p < 0.001$).
##Significantly different from the NH group (Welch's ANOVA, $p < 0.001$; post-hoc Tamhane test, $p < 0.01$).
§Significantly different from the S-CP group (Welch's ANOVA, $p < 0.001$; post-hoc Tamhane test, $p < 0.05$).
‡Significantly different from the SG group (Welch's ANOVA, $p < 0.001$; post-hoc Tamhane test, $p < 0.001$).
‡‡Significantly different from the NG group (Welch's ANOVA, $p < 0.001$; post-hoc Tamhane test, $p < 0.05$).
‡Significant difference between smokers and nonsmokers with the same periodontal status (Welch's ANOVA, $p < 0.001$; post-hoc Tamhane test, $p < 0.05$).
N-CP, nonsmokers with chronic periodontitis; NG, nonsmokers with gingivitis; N-GAgP, nonsmokers with generalized aggressive periodontitis; NH, periodontally healthy nonsmokers; S-CP, smokers with chronic periodontitis; SG, smokers with gingivitis; S-GAgP, smokers with generalized aggressive periodontitis; SH, periodontally healthy smokers.

Table 3. Correlations of the gingival crevicular fluid interleukin-8 (IL-8) and lipoxin A_4 (LxA_4) levels with clinical parameters of the sampling sites of smokers and nonsmokers

	IL-8 (pg/mL) concentration		IL-8 (pg/30 s) total amount		LxA_4 (pg/mL) concentration		LxA_4 (pg/30 s) total amount	
Clinical parameter	Smoker	Non smoker	Smoker	Non smoker	Smoker	Non smoker	Smoker	Non smoker
PI	$R = 0.54$	$R = 0.43$	$R = 0.52$	$R = 0.56$	$R = -0.30$	$R = 0.36$	NS	NS
	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.05$	$p < 0.05$		
GI	$R = 0.61$	$R = 0.55$	$R = 0.68$	$R = 0.64$	$R = -0.31$	$R = 0.36$	$R = 0.27$	NS
	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.05$	$p < 0.05$	$p < 0.05$	
Probing pocket depth	$R = 0.84$	$R = 0.71$	$R = 0.93$	$R = 0.86$	$R = -0.82$	$R = 0.62$	$R = 0.58$	$R = 0.29$
	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.05$
Clinical attachment level	$R = 0.82$	$R = 0.72$	$R = 0.92$	$R = 0.87$	$R = -0.81$	$R = 0.64$	$R = 0.47$	$R = 0.30$
	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.05$
BOP	$R = 0.82$	$R = 068$	$R = 0.90$	$R = 083$	$R = -0.76$	$R = 060$	$R = 0.52$	$R = 0.25$
	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.05$
Gingival crevicular fluid	$R = 0.47$	$R = 0.51$	$R = 0.86$	$R = 0.82$	$R = -0.57$	$R = 0.59$	$R = 0.44$	$R = 0.59$
	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$

Values were calculated using Pearson correlation analysis.
BOP, bleeding on probing; GI, gingival index; PI, plaque index.

Table 4. Correlations between the levels of gingival crevicular fluid interleukin-8 (IL-8) and lipoxin A_4 (LxA_4) in the sampling sites of smokers and nonsmokers

	IL-8 (pg/mL) Concentration		IL-8 (pg/30 s) Total amount	
	Smoker	Nonsmoker	Smoker	Nonsmoker
LxA_4 (pg/mL) concentration	$R = -0.78$ $p < 0.01$	$R = -0.53$ $p < 0.01$	$R = -0.82$ $p < 0.01$	$R = -0.67$ $p < 0.01$
LxA_4 (pg/30 s) total amount	$R = -0.81$ $p < 0.01$	$R = -0.52$ $p < 0.01$	$R = -0.57$ $p < 0.01$	$R = -0.36$ $p < 0.05$

Values were calculated using Pearson correlation analysis.

(32) reported similar results for IL-8 in gingival supernatant. In contrast, Giannopoulou *et al*. (19) and Ertuğrul *et al*. (33) found total gingival crevicular fluid IL-8 values to be higher among patients with generalized aggressive periodontitis than among patients with chronic periodontitis.

When gingival crevicular fluid IL-8 levels were evaluated according to smoking status in the present study, the concentrations and total amounts of gingival crevicular fluid IL-8 were significantly higher in smokers with chronic and generalized aggressive periodontitis than in nonsmokers with similar periodontal health statuses, and slightly higher among smokers with gingivitis and healthy periodontal status than among nonsmokers with gingivitis and healthy periodontal status, although the differences between the latter groups were not statistically significant. Various explanations have been put forward regarding the effects of smoking on gingival crevicular fluid IL-8 levels, which has previously been shown to vary according to the type of periodontal disease (gingivitis, chronic periodontitis or aggressive periodontitis) (18,19,34,35). A recent study by Kaval *et al*. (36) found that apart from a clear difference in salivary cotinine concentrations between smokers and nonsmokers with chronic periodontitis, other biochemical parameters, including gingival crevicular fluid IL-8, were similar for both groups of patients. An earlier study by Kamma *et al*. (34) also reported similar gingival crevicular fluid IL-8 levels for smokers and nonsmokers with aggressive periodontitis. In contrast to these findings, Tymkiw *et al*. (21) reported gingival crevicular fluid IL-8 levels to be significantly higher in smokers with chronic periodontitis than in nonsmokers with chronic periodontitis. Giannopoulou *et al*. (19) also found IL-8 levels in gingival crevicular fluid of smokers to be higher than in nonsmokers with a similar periodontal status.

The significantly higher gingival crevicular fluid IL-8 levels observed in this study in smokers with aggressive and chronic periodontitis compared with nonsmokers with the same periodontal status may be an indication of the effects of smoking on PMN chemotaxis and migration in the periodontium, especially under diseased conditions (22–24). Alternatively, given that smoking has been shown to have a direct negative effect on the progression of periodontal disease, as well as on the treatment of this disease, the findings described above could be a result of changes in IL-8 expression in diseased periodontal tissue caused by smoking.

Our study found a positive correlation between gingival crevicular fluid IL-8 values and clinical assessments in both smokers and nonsmokers. This correlation is an indication that the release of IL-8 from inflamed periodontal tissue is dependent upon the severity and extent of periodontal destruction and is in line with previous studies showing a connection between gingival crevicular fluid IL-8 levels and the severity of periodontal disease (19,31–33). Furthermore, the increase in gingival crevicular fluid IL-8 levels observed in subjects with aggressive periodontitis, although not statistically significant, suggests that increases in gingival crevicular fluid IL-8 content may be related to damage associated with the severe level of destruction caused by this type of periodontal disease. The lack of a significant difference between the gingival crevicular fluid IL-8 levels of subjects with chronic periodontitis and those with generalized aggressive periodontitis may be explained by the small study population and/or the similarity in the clinical assessments of the different periodontitis groups.

Our study also found gingival crevicular fluid LxA_4 levels to be significantly lower among smokers with generalized aggressive periodontitis than among smokers with chronic periodontitis, and slightly lower among nonsmokers with generalized aggressive periodontitis than among nonsmokers with chronic periodontitis. The total amounts and concentrations of gingival crevicular fluid LxA_4 were also lower among smokers with gingivitis than among smokers with periodontitis, and the concentrations of gingival crevicular fluid LxA_4 were significantly lower among nonsmokers with gingivitis than among nonsmokers with periodontitis.

When LxA_4 was evaluated according to smoking status, the concentrations and total amounts of gingival crevicular fluid LxA_4 were found to be significantly lower among smokers with generalized aggressive periodontitis and chronic periodontitis than among nonsmokers with the same periodontal status. The levels of gingival crevicular fluid LxA_4 were also lower among smokers with gingivitis than among nonsmokers with gingivitis, but the difference was not statistically significant.

Given their role in leukocyte trafficking, positive and negative endogenous mediators of inflammation could be referred to as local 'stop' and 'go' signals, with LxA_4 among the most important 'stop' signs (3,10,37). Both experimental and human studies have identified Lx molecules in a variety of tissues and inflammatory exudates (12,14,38–42). Several studies have reported LxA_4 to inhibit PMN chemotaxis, adhesion to endothelial cells and transmigration across endothelial and epithelial cells *in vitro*

(12,38,41,43,44). However, only a limited number of studies have examined the effects of LxA_4 on the pathogenesis of periodontal disease; moreover, studies aimed at clarifying the role of resolution mediators in the disease process have been conducted using animal models. For example, Serhan *et al*. (9) found that the over-expression of 15-lipoxygenase Type I in rabbits increased the levels of endogenous LxA_4, thereby protecting the host from developing periodontal disease. The authors suggested that there is an association between the over-expression of LxA_4 and tissue degradation and bone loss as a result of dampened PMN expression, and that enhanced anti-inflammatory status is an active process (9). In another set of experiments, Pouliot *et al*. (45) introduced the periodontal pathogen *Porphyromonas gingivalis* into murine dorsal air pouches and then triggered Lx release through the administration of metabolically stable analogous of Lx and aspirin. The authors reported that Lx blocked PMN traffic into the dorsal pouch cavity, providing strong support to the notion that Lx can protect against periodontitis by limiting PMN recruitment and PMN-mediated tissue injury (45). In a human-based *in vitro* study, administration of resolvin E1 and LxA_4 to PMN and monocytes obtained from the blood of subjects with localized aggressive periodontitis decreased PMN superoxide production in response to tumor necrosis factor-alpha and bacterial peptide N-formyl-methionyl-leucyl-phenylalanine by 80% and repaired compromised macrophage phagocytic activity (46). In another recent study, Börgeson *et al*. (15) found that LxA_4 antagonized *P. gingivalis*-induced PMN and platelet activation in human blood; the authors suggested that this finding could contribute to possible new strategies for preventing and treating periodontitis and other inflammatory disorders.

To the best of our knowledge, no previously published study has directly examined differences in gingival crevicular fluid LxA_4 levels between healthy and diseased periodontal tissue in conjunction with the effects of smoking on these tissue levels; however, several studies have examined the interaction of smoking and LxA_4 in lung tissue. LxA_4 has been found to play a role as an anti-inflammatory mediator in chronic obstructive pulmonary disease (COPD), which is characterized by an enhanced inflammatory response to smoking that persists despite quitting. However, the findings regarding COPD are inconsistent, with variations in response among tissue type. For example, one study reported elevated LxA_4 levels in the sputum of patients with COPD (47), whereas another reported reduced LxA_4 levels in exhaled breath condensate (48) and yet another reported similar LxA_4 levels in lung tissue samples of current and former smokers with COPD, regardless of inflammation and disease status (49). Despite these contradictory results, LXA_4 treatment is currently considered to be an important and unique therapeutic approach to reducing inflammation in COPD (50).

In our study, a negative correlation was found between clinical assessments and LxA_4 levels, indicating that LxA_4 may have the capacity to limit host damage and promote the resolution of inflammation in diseased tissue. At the same time, the significant differences between LxA_4 levels of smokers with aggressive and chronic periodontitis, compared with those of nonsmokers with the same periodontal status, may be linked to the pathophysiologic effects of smoking on inflammatory cell action, inflammatory mediator production and the capacity to deregulate the normal response of periodontal tissues (17,19,22).

In the present study, negative correlations were found between the total amounts and concentrations of IL-8 and the total amounts and concentrations of LxA_4 in the gingival crevicular fluid of both smokers and nonsmokers with periodontal disease; however, the correlation was much stronger among smokers than among nonsmokers. LxA_4 has been identified as a mediator in protecting the host against PMN-mediated tissue injury by limiting the production of the chemokines that direct PMN trafficking (38,43,51). Both *in vitro* and *in vivo* studies have suggested that LxA_4 inhibits IL-8 production in various tissues (52), cells (39,53–55) and biological fluids (51,56). For example, one study (57) found relatively high concentrations of IL-8 in the sputum of patients with severe asthma and relatively low concentrations of LxA_4 in the sputum of patients with mild asthma, despite chronic airway inflammation in both groups. A similar study (58) reported decreases in sputum IL-8 concentrations accompanied by increases in sputum LxA_4 concentrations of patients being treated with antibiotics for chronic bronchial infection. All of these results are in line with those of our study, supporting our assumption that the increases observed in IL-8, in conjunction with decreases in LxA_4, are an indication of the anti-inflammatory effect of lipid mediators. Furthermore, the much stronger relationship between IL-8 and LxA_4 observed in smokers compared with nonsmokers may be attributed to the connection between smoking and increases in cytokine release and accumulation of inflammatory cells at disease-affected sites (17,20).

The findings of the present study are limited by the fact that cotinine levels were not measured. Considering that the amounts of IL-8 have been suggested to increase in line with increases in the period of stimulation with nicotine and cigarette smoke extracts (20,59,60), future studies should assay cotinines, as well as IL-8, to provide a better picture of the dose-dependent effect of smoking on IL-8 expression (34,60).

Conclusion

The complex effects of smoking on periodontal and oral diseases, and the mechanisms that mediate these diseases, are still considerably important. Given our findings on IL-8 and LxA_4, smoking effectively altered the levels of neutrophil related pro-inflammatory (IL-8) and anti-inflammatory (LxA_4) mediators, especially in

individuals with aggressive and chronic periodontitis. IL-8 and LxA_4 molecules play important roles in determining neutrophil function. On the other hand, evaluating the levels of Lx, one of the body's own natural anti-inflammatory mediators, could be useful in clarifying the mechanism of periodontal disease progression in individuals predisposed to increased periodontal destruction. Within the limits of this study, we believe that smoking might create an apparent alteration in the local host response of individuals and/or sites with severe periodontal destruction, and hereby it is important to identify the biological mediators related to progressive periodontal destruction, in order to formulate recommendations for preventing periodontal disease progression and developing strategies for its treatment.

References

1. Garlet GP. Destructive and protective roles of cytokines in periodontitis: a re-appraisal from host defense and tissue destruction viewpoints. *J Dent Res* 2010;**89**:1349–1363.
2. Nussbaum G, Shapira L. How has neutrophil research improved our understanding of periodontal pathogenesis? *J Clin Periodontol* 2011;**38**:49–59.
3. Van Dyke TE. Proresolving lipid mediators: potential for prevention and treatment of periodontitis. *J Clin Periodontol* 2011;**38**:119–125.
4. Freire MO, Van Dyke TE. Natural resolution of inflammation. *Periodontol 2000* 2013;**63**:149–164.
5. Shelburne CE, Coopamah MD, Sweier DG, An FY, Lopatin DE. HtpG, the *Porphyromonas gingivalis* HSP-90 homologue, induces the chemokine CXCL8 in human monocytic and microvascular vein endothelial cells. *Cell Microbiol* 2007;**9**:1611–1619.
6. Kantarci A, Van Dyke TE. Resolution of inflammation in periodontitis. *J Periodontol* 2005;**76**:2168–2174.
7. Kantarci A, Van Dyke TE. Lipoxin signaling in neutrophils and their role in periodontal disease. *Prostaglandins Leukot Essent Fatty Acids* 2005;**73**:289–299.
8. Kantarci A, Van Dyke TE. Lipoxins in chronic inflammation. *Crit Rev Oral Biol Med* 2003;**14**:4–12.
9. Serhan CN, Jain A, Marleau S *et al.* Reduced inflammation and tissue damage in transgenic rabbits overexpressing 15-lipoxygenase and endogenous anti-inflammatory lipid mediators. *J Immunol* 2003;**171**:6856–6865.
10. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class switching during acute inflammation: signals in resolution. *Nat Immunol* 2001;**2**:612–619.
11. Kantarci A, Hasturk H, Van Dyke TE. Host-mediated resolution of inflammation in periodontal diseases. *Periodontol 2000* 2006;**40**:144–163.
12. Godson C, Mitchell S, Harvey K, Petasis NA, Hogg N, Brady HR. Cutting edge: lipoxins rapidly stimulate nonphlogistic phagocytosis of apoptotic neutrophils by monocyte-derived macrophages. *J Immunol* 2000;**164**:1663–1667.
13. Kantarci A, Oyaizu K, Van Dyke TE. Neutrophil-mediated tissue injury in periodontal disease pathogenesis: findings from localized aggressive periodontitis. *J Periodontol* 2003;**74**:66–75.
14. Weinberger B, Quizon C, Vetrano AM, Archer F, Laskin JD, Laskin DL. Mechanisms mediating reduced responsiveness of neonatal neutrophils to lipoxin A4. *Pediatr Res* 2008;**64**:393–398.
15. Börgeson E, Lönn J, Bergström I *et al.* Lipoxin A_4 inhibits *Porphyromonas gingivalis*-induced aggregation and reactive oxygen species production by modulating neutrophil-platelet interaction and CD11b expression. *Infect Immun* 2011;**79**:1489–1497.
16. Van Dyke TE, Hasturk H, Kantarci A *et al.* Proresolving Nanomedicines Activate Bone Regeneration in Periodontitis. *J Dent Res* 2015;**94**:148–156.
17. Palmer RM, Wilson RF, Hasan AS, Scott DA. Mechanisms of action of environmental factors–tobacco smoking. *J Clin Periodontol* 2005;**32**:180–195.
18. Giannopoulou C, Cappuyns I, Mombelli A. Effect of smoking on gingival crevicular fluid cytokine profile during experimental gingivitis. *J Clin Periodontol* 2003;**30**:996–1002.
19. Giannopoulou C, Kamma JJ, Mombelli A. Effect of inflammation, smoking and stress on gingival crevicular fluid cytokine level. *J Clin Periodontol* 2003;**30**:145–153.
20. Mahanonda R, Sa-Ard-Iam N, Eksomtramate M *et al.* Cigarette smoke extract modulates human beta-defensin-2 and interleukin-8 expression in human gingival epithelial cells. *J Periodontal Res* 2009;**44**:557–564.
21. Tymkiw KD, Thunell DH, Johnson GK *et al.* Influence of smoking on gingival crevicular fluid cytokines in severe chronic periodontitis. *J Clin Periodontol* 2011;**38**:219–228.
22. Petropoulos G, McKay IJ, Hughes FJ. The association between neutrophil numbers and interleukin-1alpha concentrations in gingival crevicular fluid of smokers and non-smokers with periodontal disease. *J Clin Periodontol* 2004;**31**:390–395.
23. Güntsch A, Erler M, Preshaw PM, Sigusch BW, Klinger G, Glockmann E. Effect of smoking on crevicular polymorphonuclear neutrophil function in periodontally healthy subjects. *J Periodontal Res* 2006;**41**:184–188.
24. Barbour SE, Nakashima K, Zhang JB *et al.* Tobacco and smoking: environmental factors that modify the host response (immune system) and have an impact on periodontal health. *Crit Rev Oral Biol Med* 1997;**8**:437–460.
25. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* 1999;**4**:1–6.
26. Silness J, Löe H. Periodontal disease in pregnancy. II. correletion between oral hygiene and periodontal condition. *Acta Odontol Scand* 1964;**22**:121–135.
27. Löe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand* 1963;**21**:533–551.
28. Tüter G, Ozdemir B, Kurtiş B, Serdar M, Yücel AA, Ayhan E. Short term effects of non-surgical periodontal treatment on gingival crevicular fluid levels of tissue plasminogen activator (t-PA) and plasminogen activator inhibitor 2 (PAI-2) in patients with chronic and aggressive periodontitis. *Arch Oral Biol* 2013;**58**:391–396.
29. Curtis MA, Griffiths GS, Price SJ, Coulthurst SK, Johnson NW. The total protein concentration of gingival crevicular fluid. Variation with sampling time and gingival inflammation. *J Clin Periodontol* 1988;**15**:628–632.
30. Konopka L, Pietrzak A, Brzezińska-Błaszczyk E. Effect of scaling and root planing on interleukin-1β, interleukin-8 and MMP-8 levels in gingival crevicular fluid from chronic periodontitis patients. *J Periodontal Res* 2012;**47**:681–688.
31. Liu RK, Cao CF, Meng HX, Gao Y. Polymorphonuclear neutrophils and their mediators in gingival tissues from generalized aggressive periodontitis. *J Periodontol* 2001;**72**:1545–1553.
32. Rescala B, Rosalem W Jr, Teles RP *et al.* Immunologic and microbiologic profiles of chronic and aggressive periodontitis subjects. *J Periodontol* 2010;**81**:1308–1316.
33. Ertugrul AS, Sahin H, Dikilitas A, Alpaslan N, Bozoglan A. Comparison of CCL28, interleukin-8, interleukin-1β and tumor necrosis factor-alpha in subjects with gingivitis, chronic periodontitis and generalized aggressive periodontitis. *J Periodontal Res* 2013;**48**:44–51.

34. Kamma JJ, Giannopoulou C, Vasdekis VG, Mombelli A. Cytokine profile in gingival crevicular fluid of aggressive periodontitis: influence of smoking and stress. *J Clin Periodontol* 2004;**31**:894–902.
35. Ozmeric N, Bal B, Balos K, Berker E, Bulut S. The correlation of gingival crevicular fluid interleukin-8 levels and periodontal status in localized juvenile periodontitis. *J Periodontol* 1998;**69**: 1299–1304.
36. Kaval B, Renaud DE, Scott DA, Buduneli N. The role of smoking and gingival crevicular fluid markers on coronally advanced flap outcomes. *J Periodontol* 2014;**85**:395–405.
37. Serhan CN, Hamberg M, Samuelsson B. Lipoxins: novel series of biologically active compounds formed from arachidonic acid in human leukocytes. *Proc Natl Acad Sci USA* 1984;**81**:5335–5339.
38. Hachicha M, Pouliot M, Petasis NA, Serhan CN. Lipoxin (LX)A4 and aspirin-triggered 15-epi-LXA4 inhibit tumor necrosis factor 1alpha-initiated neutrophil responses and trafficking: regulators of a cytokine-chemokine axis. *J Exp Med* 1999;**189**:1923–1930.
39. Wu SH, Liao PY, Dong L, Chen ZQ. Signal pathway involved in inhibition by lipoxin A(4) of production of interleukins induced in endothelial cells by lipopolysaccharide. *Inflamm Res* 2008;**57**: 430–437.
40. Edenius C, Stenke L, Lindgren JA. On the mechanism of transcellular lipoxin formation in human platelets and granulocytes. *Eur J Biochem* 1991;**199**: 401–419.
41. Leonard MO, Hannan K, Burne MJ *et al*. 15-Epi-16-(para-fluorophenoxy)-lipoxin A(4)-methyl ester, a synthetic analogue of 15-epi-lipoxin A(4), is protective in experimental ischemic acute renal failure. *J Am Soc Nephrol* 2002;**13**: 1657–1662.
42. Medeiros R, Rodrigues GB, Figueiredo CP *et al*. Molecular mechanisms of topical anti-inflammatory effects of lipoxin A (4) in endotoxin-induced uveitis. *Mol Pharmacol* 2008;**74**:154–161.
43. Filep JG, Khreiss T, József L. Lipoxins and aspirin-triggered lipoxins in neutrophil adhesion and signal transduction. *Prostaglandins Leukot Essent Fatty Acids* 2005;**73**:257–262.
44. Colgan SP, Serhan CN, Parkos CA, Delp-Archer C, Madara JL. Lipoxin A4 modulates transmigration of human neutrophils across intestinal epithelial monolayers. *J Clin Invest* 1993;**92**:75–82.
45. Pouliot M, Clish CB, Petasis NA, Van Dyke TE, Serhan CN. Lipoxin A(4) analogues inhibit leukocyte recruitment to *Porphyromonas gingivalis*: a role for cyclooxygenase-2 and lipoxins in periodontal disease. *Biochemistry* 2000;**39**: 4761–4768.
46. Fredman G, Oh SF, Ayilavarapu S, Hasturk H, Serhan CN, Van Dyke TE. Impaired phagocytosis in localized aggressive periodontitis: rescue by Resolvin E1. *PLoS ONE* 2011;**6**:e24422.
47. Vachier I, Bonnans C, Chavis C *et al*. Severe asthma is associated with a loss of LX4, an endogenous anti-inflammatory compound. *J Allergy Clin Immunol* 2005;**115**:55–60.
48. Fritscher LG, Post M, Rodrigues MT *et al*. Profile of eicosanoids in breath condensate in asthma and COPD. *J Breath Res* 2012;**6**:026001.
49. Noguera A, Gomez C, Faner R *et al*. An investigation of the resolution of inflammation (catabasis) in COPD. *Respir Res* 2012;**13**:101.
50. Bozinovski S, Uddin M, Vlahos R *et al*. Serum amyloid A opposes lipoxin A_4 to mediate glucocorticoid refractory lung inflammation in chronic obstructive pulmonary disease. *Proc Natl Acad Sci USA* 2012;**109**:935–940.
51. József L, Zouki C, Petasis NA, Serhan CN, Filep JG. Lipoxin A4 and aspirin-triggered 15-epi-lipoxin A4 inhibit peroxynitrite formation, NF-kappa B and AP-1 activation, and IL-8 gene expression in human leukocytes. *Proc Natl Acad Sci USA* 2002;**99**:13266–13271.
52. Bonnans C, Gras D, Chavis C *et al*. Synthesis and anti-inflammatory effect of lipoxins in human airway epithelial cells. *Biomed Pharmacother* 2007;**61**:261–267.
53. Decker Y, McBean G, Godson C. Lipoxin A4 inhibits IL-1beta-induced IL-8 and ICAM-1 expression in 1321N1 human astrocytoma cells. *Am J Physiol Cell Physiol* 2009;**296**:1420–1427.
54. Baker N, O'Meara SJ, Scannell M, Maderna P, Godson C. Lipoxin A4: anti-inflammatory and anti-angiogenic impact on endothelial cells. *J Immunol* 2009;**182**:3819–3826.
55. Sodin-Semrl S, Taddeo B, Tseng D, Varga J, Fiore S. Lipoxin A4 inhibits IL-1 beta-induced IL-6, IL-8, and matrix metalloproteinase-3 production in human synovial fibroblasts and enhances synthesis of tissue inhibitors of metalloproteinases. *J Immunol* 2000;**164**:2660–2666.
56. Shimizu S, Ogawa T, Seno S, Kouzaki H, Shimizu T. Pro-resolution mediator lipoxin A4 and its receptor in upper airway inflammation. *Ann Otol Rhinol Laryngol* 2013;**122**:683–689.
57. Bonnans C, Vachier I, Chavis C, Godard P, Bousquet J, Chanez P. Lipoxins are potential endogenous antiinflammatory mediators in asthma. *Am J Respir Crit Care Med* 2002;**165**:1531–1535.
58. Chiron R, Grumbach YY, Quynh NV, Verriere V, Urbach V. Lipoxin A(4) and interleukin-8 levels in cystic fibrosis sputum after antibiotherapy. *J Cyst Fibros* 2008;**7**:463–468.
59. Kashiwagi Y, Yanagita M, Kojima Y, Shimabukuro Y, Murakami S. Nicotine up-regulates IL-8 expression in human gingival epithelial cells following stimulation with IL-1β or *P. gingivalis* lipopolysaccharide via nicotinic acetylcholine receptor signalling. *Arch Oral Biol* 2012;**57**:483–490.
60. An N, Andrukhov O, Tang Y *et al*. Effect of nicotine and *Porphyromonas gingivalis* lipopolysaccharide on endothelial cells *in vitro*. *PLoS ONE* 2014;**9**:e96942.