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Effects of periodontal therapy on GCF cytokines in generalized aggressive periodontitis subjects

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Abstract

Aim—To examine changes in levels of gingival crevicular fluid (GCF) cytokines, after periodontal therapy of generalized aggressive periodontitis (GAgP).

Materials and Methods—Twenty five periodontally healthy and 24 GAgP subjects had periodontal clinical parameters measured and gingival crevicular fluid (GCF) samples collected from up to 14 sites/subject. GCF samples were analyzed using multiplex bead immunoassay for: GM-CSF, IFN- γ , IL-10, IL-1 β , IL-2, IL-6 and TNF- α . Aggressive periodontitis subjects were randomly assigned to either scaling and root planing (SRP) alone or SRP plus systemic amoxicillin (500 mg) and metronidazole (400 mg) 3 times a day for 14 days. Clinical parameters and GCF cytokines were re-measured 6 months after treatment. Differences over time were analyzed using the Wilcoxon test and between groups using the Mann-Whitney test.

Results—Significant reductions in GCF GM-CSF, IL-1 β and the ratio IL-1 β /IL-10 and increases in GCF IL-6 were detected after therapy. The mean change in GCF cytokines did not differ significantly between groups.

Conclusions—Periodontal therapy improved GCF cytokine profiles by lowering IL-1 β and increasing IL-10 levels. The reduction in GCF GM-CSF after therapy implicates this cytokine in the pathogenesis of GAgP. There was no difference between therapies in changes of GCF cytokines.

Keywords

periodontal disease; cytokines; gingival crevicular fluid; periodontal therapy

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Conflict of Interest

The authors declare that they have no conflict of interests.

INTRODUCTION

Studies examining biomarkers in gingival crevicular fluid (GCF) have implicated several host derived mediators in the pathogenesis of periodontal diseases. These studies have focused on biomarkers with functions that fit our understanding of the pathological mechanisms involved in periodontal disease initiation and progression including pro-inflammatory cytokines, T helper 1 (Th1) cytokines and anti-inflammatory cytokines.

Pro-inflammatory cytokines such as interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α) are released primarily by macrophages after bacterial infection or tissue injury (Dinarello, 1987). When released in high concentrations these cytokines can stimulate the production and release of other inflammatory mediators such as IL-6, matrix metalloproteinases (MMPs) and prostaglandin E2 (PGE₂) (Dinarello, 2007). IL-1 β and TNF- α are also potent inducers of bone resorption and inhibitors of bone formation (Stashenko et al., 1987a, Stashenko et al., 1987b). Several reports have indicated that GCF IL-1 β is elevated in periodontitis patients, when compared to healthy and gingivitis patients (Hou et al., 1995, Engebretson et al., 2002, Stashenko et al., 1991), is higher in active versus inactive sites (Reinhardt et al., 2010) and declines after periodontal treatment (Engebretson et al., 2002, Zhong et al., 2007, Rosalem et al., 2011, Hou et al., 1995, Al-Shammari et al., 2001). Furthermore, soluble antagonists to interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α) were capable of reducing loss of connective tissue attachment and alveolar bone in non-human primates (Delima et al., 2001). Interleukin-6 is often secreted together with other pro-inflammatory cytokines during the induction of acute phase reactions. Early reports indicated that GCF levels of IL-6 were elevated during periodontal disease progression in chronic (Geivellis et al., 1993) and in refractory periodontitis (Lee et al., 1995). A recent randomized, placebo-controlled study on the impact of adjunctive subantimicrobial-dose doxycycline (SDD) demonstrated SDD was associated with a higher decrease in GCF IL-6 compared to the placebo group (Emingil et al.). These findings suggest that IL-6 is involved in the inflammation induced tissue destruction of the periodontium. Interleukin-2 and interferon- γ (IFN- γ) are cytokines released by the Th1 subset of CD4 T-helper cells. Due to the potent pro-inflammatory properties of IFN- γ several authors have proposed a role for a Th1-type response during periodontal disease activity (Dutzan et al., 2009, Stashenko et al., 2007). In fact, IFN- γ has been associated with progressing periodontal lesions of chronic periodontitis (Dutzan et al., 2009, Alpagot et al., 2003). Higher levels of GCF IL-2 have also been reported in active sites of refractory periodontitis subjects compared to non-progressing sites (Lee et al., 1995). However, studies on the effects of periodontal therapy on Th1 GCF cytokines have been inconclusive. A recent study has reported statistically significant reductions as a result of periodontal mechanical treatment in GCF IFN- γ and IL-2 (Thunell et al., 2010) while another demonstrated a trend for an increase in GCF levels of IFN- γ after periodontal therapy (Del Peloso Ribeiro et al., 2008). In addition, Rosalem et al. 2011 could not detect any significant changes in GCF IFN- γ in response to periodontal treatment.

Studies using IL-10 knockout mice have implicated this anti-inflammatory cytokine in the pathogenesis of periodontal diseases. Sasaki et al., 2004, 2008 demonstrated that these animals are highly susceptible to alveolar bone destruction induced by *Porphyromonas gingivalis* infection. This immunoregulatory cytokine can inhibit a series of pro-inflammatory signals and has also been involved in the suppression of MMPs and in the stimulation of osteoprotegerin (OPG), an inhibitor of bone resorption (Garlet, 2010). Studies on GCF levels of IL-10 have also resulted in conflicting findings. Gamonal et al., 2000 reported a decrease in the total amounts of GCF IL-10 as a result of periodontal therapy, while Del Peloso Ribeiro et al. 2008 found a statistically significant increase in GCF IL-10 after mechanical debridement. Further, Goutoudi et al., 2004 could not find differences in

the GCF levels of IL-10 between periodontally diseased and non-diseased sites and reported no changes as a result of treatment.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is produced primarily by monocytes/macrophages, fibroblasts and endothelial cells and plays an essential role in normal neutrophil development. This cytokine has been shown to prolong the persistence of neutrophils in gingival tissues from chronic periodontitis subjects by reducing their apoptosis (Gamonal et al., 2003). However, the presence of GM-CSF in GCF has not been extensively studied and the only report that examined this inflammatory mediator in GCF samples from periodontitis subjects did not find differences between healthy and diseased sites (Thunell et al., 2010). In addition, the authors could not find a change in GCF GM-CSF levels as a consequence of mechanical periodontal therapy.

We have previously analyzed clinical parameters, microbial data and levels of GCF cytokines in generalized aggressive periodontitis (GAgP) subjects (Teles et al., 2010). This cross-sectional study demonstrated that GAgP subjects had statistically significantly higher GCF levels of IL-1 β , granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-1 β /IL-10 ratio compared to periodontally healthy subjects. We concluded that GAgP subjects were characterized by a higher IL-1 β /IL-10 ratio than periodontally healthy subjects, suggesting an imbalance between pro- and anti-inflammatory cytokines in aggressive periodontitis. The goal of the present study was to follow up the cross-sectional study by an intervention study that would determine if periodontal treatment would change the GCF cytokine profile in aggressive periodontitis to one more similar to that observed in periodontal health. Thus, changes in levels of GCF IL-1 β , TNF- α , IL-6, IFN- γ , IL-2, IL-10 and GM-CSF were assessed in GAgP subjects as a result of two forms of periodontal therapy.

Materials and Methods

Subject population and clinical monitoring

Twenty five periodontally healthy and thirty GAgP subjects were recruited at the Guarulhos University periodontal clinic (Guarulhos, SP, Brazil). The Guarulhos University's Ethics Committee approved the study protocols including the periodontal examination and the taking of GCF samples. Each subject read and signed an informed consent form before entering the study. To be included in the study, the periodontally healthy subjects had to be 18 and < 30 years of age, have at least 24 natural teeth and no probing pocket depth (PD) and clinical attachment level (CAL) > 3 mm and have less than 20% of the sites with bleeding on probing (BOP). The GAgP subjects had to be $\geq$ 30 years of age, have at least 20 natural teeth and a minimum of six incisors and/or first molars with at least one site with PD and CAL $\geq$ 5 mm, as well as a minimum of six teeth other than first molars and incisors also presenting at least one site with PD and CAL $\geq$ 5 mm.

The diagnosis of GAgP was based on the criteria set forth by the American Academy of Periodontology (Armitage, 1999) and required familial aggregation; i.e. at least one other family member either presenting or with a history of periodontal disease. Subjects were excluded if they had any systemic condition that would influence the course of periodontal disease, and medical conditions that would require antibiotic prophylaxis for dental procedures. Individuals who smoked, had taken antibiotics in the previous 6 months, had received any previous subgingival therapy or were either pregnant or nursing were also excluded.

Bleeding on probing (0 or 1), PD (mm) and CAL (mm) were measured at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual) for all teeth

present (excluding third molars), for a maximum of 168 sites per subject, by a calibrated examiner (M.J.M). PD and CAL were recorded to the nearest millimeter using a North Carolina periodontal probe (Hu-friedy, Chicago, IL, USA).

Experimental design

In this double-blinded, randomized clinical trial, GAgP subjects were randomly assigned to one of the following treatment groups: SRP + placebo or SRP + systemic antibiotics (SRP + MET and AMX). The antibiotic regimen prescribed was: metronidazole (MET) 400 mg and amoxicillin (AMX) 500 mg three times a day (t.i.d.) for 14 days. Subjects in the SRP group received two placebo capsules t.i.d. for 14 days. All subjects were instructed to rinse for 1 min with 15 ml of 0.12% chlorhexidine (CHX) solution twice a day for 60 days. The antibiotic and placebo regimens and the chemical plaque control started immediately after the first session of mechanical instrumentation. Clinicians and study subjects were blinded to treatment assignment during the study. All GAgP subjects received full-mouth supragingival scaling, instruction in oral hygiene and full-mouth SRP performed under local anesthesia in up to six appointments lasting approximately 1 h each. Mechanical therapy was completed within 14 days of the baseline visit and SRP was performed by a single trained periodontist using manual instruments (M.J.M). Clinical monitoring for GAgP subjects was repeated 6 months post-therapy.

Gingival Crevicular Fluid sampling

Gingival crevicular fluid (GCF) samples were obtained from the mesiobuccal site of every tooth present (excluding third molars) in two randomly selected contra-lateral quadrants (up to 14 sites per subject). Following isolation of the site with cotton rolls, supragingival plaque was removed, the area air dried and a 30-second GCF sample collected with filter strips (Periopapers, Interstate Drug Exchange, Amityville, NY, USA). Periopaper strips were gently inserted into the orifice of the periodontal pocket, 1–2mm subgingivally. Gingival crevicular fluid volume was determined using a Periotron 8000 (Oraflow Inc., Plainview, NY, USA), calibrated following the protocol described by (Chapple et al., 1999). Samples were immediately placed in Eppendorf tubes on ice, transported to the laboratory and stored at –80°C. GCF samples were collected before clinical measurements at baseline and 6 months after treatment. Samples visibly contaminated with blood were discarded. All GCF samples were shipped frozen to the Forsyth Institute, Cambridge, MA for analysis.

Quantification of cytokines using multiplexed bead immunoassay (Luminex)

Cytokine levels were determined using a high-sensitivity human cytokine 7-plex Millipore kit (Millipore Corporation, Billerica, MA, USA). Before the assay, GCF samples were eluted using 60 µl of the assay buffer provided in the Millipore kit by vortexing for 30 min. and then centrifuging for 10 min. at 10,000 rpm. Seven cytokines: GM-CSF, IL-2, interferon- γ (IFN- γ), IL-10, IL-6, IL-1 β and TNF- α were measured. The assays were performed in 96-well filter plates following the manufacturer's instructions (for details see Teles et al. 2010). Briefly, microsphere beads coated with monoclonal antibodies against the seven different target analytes were added to the wells of a filter plate. Samples and standards (ranging from 0.13 to 2000 pg/ml for each analyte) were added and incubated overnight at 4°C. The wells were washed and a mixture of biotinylated secondary antibodies was added. After incubation, streptavidin conjugated to R-phycoerythrin was added to the beads and incubated for 30 min. After washing, sheath fluid (Luminex, MiraiBio, Alameda, CA, USA) was added to the wells and the beads were analyzed in the Luminex 100™ instrument. The concentrations of the antigens in GCF samples were estimated from the standard curve using a third-order polynomial equation and the GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA) and expressed as pg/ml. Samples below the

detection limit of the assay were recorded as zero, while samples above the upper limit of quantification of the standard curves were assigned the highest value of the curve.

Data analysis

Clinical data were obtained from 6 sites per tooth in each periodontally healthy subject at baseline and from GAgP individuals at baseline and 6 months after periodontal therapy. Mean values were calculated for each individual and averaged across subjects in each clinical group at baseline and for the two treatment groups at baseline and 6 months separately. The mean numbers and mean percentage of sites with PD >4 mm that bled on probing were also computed. Significance of changes in clinical data over time was determined using the Wilcoxon signed ranks test for each treatment group separately.

Gingival crevicular fluid levels of 7 cytokines were measured in up to 14 sites per subject. The ratio between the levels of IL-1 β /IL-10 was also calculated. The mean values for each cytokine parameter was then calculated for each subject and across subjects in each clinical group at each time point separately. Significance of changes over time in GCF biochemical parameters was determined using the Wilcoxon signed ranks test for each treatment group separately.

Clinical and cytokine baseline data were subtracted from the 6-month data to compute the changes resulting from therapy in each treatment group. Mean changes obtained by each therapy were compared using the Mann-Whitney test. Due to differences in mean PD and CAL between clinical groups, ANCOVA adjusting for baseline PD and CAL was used to confirm the significance of statistical differences obtained for unadjusted values.

To examine changes in cytokines levels at periodontal sites that exhibited AL “gain” or no change compared with sites that exhibited an increase in the AL measurement, the cytokine values of each type were averaged within each subject and then averaged across subjects in each group separately. Significant differences in mean cytokine values between the sites that gained attachment or did not change compared to those that increase in attachment were tested using the Mann-Whitney test. Similar analyses were performed for sites subset into those that decreased in PD or did not change versus those that increased in PD.

RESULTS

Subject retention

The study was conducted between July 2007 and September 2009. Out of the 30 GAgP subjects initially enrolled, GCF samples could be collected from 24 subjects at baseline and the 6-month follow-up visit: 12 in the control group (SRP + placebo) and 12 in the test group (SRP + MET and AMOX).

Clinical findings

Table 1 presents the mean ($\pm$ SD) demographic and clinical parameters for the two treatment groups at baseline and at the 6 months and for the periodontally healthy (control) group at baseline. The demographic data illustrate that the three groups were balanced for age and gender distribution. However, treatment groups had statistically significantly different mean baseline PD and CAL. The Wilcoxon test indicated that reductions in mean PD, CAL, percentage of BOP and number of pockets > 4mm that bled on probing were statistically significant in both treatment groups. When mean changes in clinical parameters were compared between treatment groups using the Mann-Whitney test, the antibiotics group had statistically significantly greater mean PD reduction and mean CAL gain compared to the placebo group (data not shown). Due to the differences in baseline mean PD and CAL the

statistical significance of comparisons between changes in clinical parameters for the two treatment groups was tested using ANCOVA adjusting for baseline PD and CAL and the adjusted values for mean PD and CAL changes remained statistically significantly different (Table 1).

GCF cytokine changes

A total of 895 GCF samples were processed as part of the study: For the samples collected from the periodontally healthy group, the frequency of detection for each of the 7 cytokines at baseline was: GM-CSF, 85%; IL-2, 83%; IFN- γ , 63%; IL-10, 99%; IL-6, 94%; IL-1 β , 99%; and TNF- α , 98%. Among GAgP subjects, the frequency of detection for each of the 7 cytokines at baseline was: GM-CSF, 79%; IL-2, 79%; IFN- γ , 69%; IL-10, 99%; IL-6, 94%; IL-1 β , 100%; and TNF- α , 99%, while the frequencies of detection six months after therapy were: GM-CSF, 83%; IL-2, 0%; IFN- γ , 43%; IL-10, 95%; IL-6, 92%; IL-1 β 96%; and TNF- α , 96%.

Gingival crevicular fluid levels of each cytokine at baseline and 6 months post-therapy in each site are highlighted in Figure 1. There was considerable variability among individual sites in the treatment response. The effect of treatment on the mean GCF cytokine levels (pg/ml) of the entire study group (N=24) can be seen in Table 2. Despite the high level of variability, significant reductions in the levels of GM-CSF and IL-1 β and increases in levels of IL-6 after treatment could be detected. The ratio IL-1 β /IL-10 also reduced significantly. The mean GCF levels of each cytokine for the control group are also presented as reference values. No statistical comparisons were conducted between the treatment groups and the control group. Figure 2 presents the entire distribution of mean values of GCF cytokines for all subjects in each treatment group separately. A certain degree of variability is still present, but it is apparent that most subjects in both groups presented decreases in mean GCF levels of GM-CSF, IL-2 and IL-1 β . Table 3 presents the mean GCF cytokine levels (pg/ml) for the two treatment groups at baseline and at the 6-month follow-up visit. There were no statistically significant differences between treatment groups. There were significant reductions in the levels of GM-CSF and the ratio IL-1 β /IL-10 in both treatment groups. In addition, in the group receiving AM there was a statistically significantly decrease in mean levels of IL-1 β . When the mean changes in levels of GCF cytokines from baseline to 6 months were compared between treatment groups using Mann-Whitney, differences were not statistically significant (data not shown).

Site level changes

When sites were subset into those that exhibited no change or “gain” in AL and compared with those that exhibited an increase in AL, there was a significant difference in mean GM-CSF values between groups. Sites that improved or did not change had a mean decrease of 2.76 ± 2.46 pg/ml versus 0.24 ± 2.76 pg/ml ($p < 0.01$). The ratio IL-1 β /IL-10 also decreased more in the sites that did not change or improved than in the sites that increased in AL (mean $\pm$ SD) 1.95 ± 2.05 compared to 0.55 ± 2.26 , respectively ($p < 0.01$). Similar significantly different changes in mean GM-CSF and IL-1 β /IL-10 ratio were observed for sites that increased or decreased in PD (data not shown).

DISCUSSION

The data presented here were obtained during a clinical trial originally designed and powered to compare SRP alone and SRP with adjunctive systemic antibiotics. The primary outcome variable was the effect of each therapy on the mean proportions of red complex species. The 3-month clinical and microbiological outcomes of the study have already been reported (Mestnik et al., 2010) and the 6-month follow-up data will be reported elsewhere

(manuscript in preparation). In the present study, GCF cytokine levels were examined as secondary outcome variables using exploratory data analysis techniques. As a first approach, we examined whether periodontal therapy, irrespective of the type, would result in changes in GCF cytokines. We then determined whether differences in efficacy could be observed between the two therapies employed. The goal was to further test our original observations of differences in GCF cytokine profiles between periodontal health and disease based on cross-sectional data by examining longitudinally the impact of therapy on GCF cytokines associated with GAgP. Due to the exploratory nature of the analysis adjustments for multiple comparisons were not included.

The clinical outcome of the randomized clinical trial provided additional evidence of a beneficial adjunctive clinical effect of the combination of amoxicillin and metronidazole when associated with mechanical debridement. The systemic antibiotic therapy not only resulted in greater mean pocket deep reduction and mean clinical attachment level gain, comparable to previously reported data (Cionca et al., 2009, Guerrero et al., 2005), but was also associated with a low mean number of residual deep pockets that bled on probing.

Our previous cross-sectional study suggested that generalized aggressive periodontitis was associated with higher GCF levels of IL-1 β , GM-CSF and the IL-1 β /IL-10 ratio than those found in a periodontally healthy subject group (Teles et al., 2010). Our current findings extend these observations by demonstrating that periodontal therapy significantly decreased GCF levels of GM-CSF, IL-1 β and the IL-1 β /IL-10 ratio. The data presented in Table 2 indicate that the levels of GCF cytokines obtained after therapy closely resembled the values for the periodontally healthy control group. In addition, the comparisons between sites that remained stable or improved to sites that “lost” clinical attachment after treatment confirmed the association between decreases in GCF GM-CSF and IL-1 β /IL-10 ratio with clinical improvements.

Interleukin-1 β has been extensively studied in GCF. In accord with our current data multiple studies demonstrated a decrease in GCF levels of IL-1 β after treatment (Engebretson et al., 2002, Rosalem et al., 2011, Zhong et al., 2007). Goutoudi et al., 2004, demonstrated a decrease in total amounts of GCF IL-1 β while IL-10 levels remained stable up to 32 weeks after surgical periodontal treatment. They concluded that periodontitis was associated with an inverse relationship between IL-1 β and IL-10. A recent paper showed that periodontal treatment of generalized aggressive periodontitis patients resulted in statistically significant reductions in GCF IL-1 β while IL-10 was unaltered (Toker et al., 2008). Our data are in agreement with these reports inasmuch we also could not find a statistically significant change in levels of IL-10 as a result of therapy. In contrast, Gamonal et al., 2000 reported a decrease in levels of GCF IL-10 as a result of scaling and root planing. Our results suggest that periodontal treatment partially reversed the perceived imbalance between the pro-inflammatory cytokine IL-1 β and the anti-inflammatory IL-10.

GM-CSF has not been extensively studied in GCF but a recent report indicated no difference in the GCF levels of this cytokine between healthy and diseased sites and no change in GCF GM-CSF content as a consequence of mechanical periodontal therapy (Thunell et al., 2010). However, the study differed considerably from ours in that: 1) the study population involved 6 subjects with generalized severe chronic periodontitis; 2) they were followed for 6–8 weeks after initial periodontal therapy; and 3) the multiplex bead immunoassay employed had a much lower sensitivity with a lower limit of detection of 15.6 pg/ml compared to 0.13 pg/ml in our study. Some of our findings were not in agreement with the current understanding of the role of IL-6 in periodontitis. This pro-inflammatory cytokine has been associated with periodontal tissue destruction and IL-6 has been proposed as a biomarker of periodontal disease progression (Geivellis et al., 1993). We anticipated that therapy would

result in reductions in their GCF levels, as recently reported by (Emingil et al., 2004). However, there is evidence that IL-6 might also act as a potent anti-inflammatory cytokine. In a murine model of periapical lesion, IL-6 knockouts and the neutralization of IL-6 using antibodies resulted in significantly higher periapical bone resorption (Balto et al., 2001).

An alternative explanation for the increase in GCF levels of IL-6 after therapy resides in the fact that GCF content might not fully reflect the levels of certain biomarkers within the inflamed periodontal tissues. Guillot et al., 1995 reported that IL-6 levels were elevated in gingival connective tissue adjacent to intrabony pockets which did not respond to mechanical therapy. However, IL-6 levels in GCF were significantly greater at sites that responded to therapy than at sites that did not. They suggested that IL-6 was localized to the inflamed tissues, resulting in a reduced rate of release into GCF.

We could not find an effect of the periodontal therapy on GCF levels of IFN- γ . Similar findings have been recently published (Rosalem et al., 2011) but conflicting data have also been reported (Thunell et al., 2010, Del Peloso Ribeiro et al., 2008). The low frequency of detection for this analyte might indicate that it is present in very low amounts in GCF. Although the significant reduction in GCF levels of IL-2 fit our understanding of its role in periodontal diseases, the low frequency of detection and our failure to detect GCF IL-2 after therapy suggest that this finding should be interpreted with caution.

Early reports indicated that the systemic use of antibiotics during periodontal treatment of subjects with “rapidly progressive periodontitis” resulted in changes in the enzyme profile of GCF that were associated with clinical improvements (Atici et al., 1998). However, recent reports on the effects of adjunctive antibiotics in the treatment of periodontitis could not demonstrate an additional impact on GCF biomarkers when compared to mechanical therapy alone (Mascarenhas et al., 2005, Machtei and Younis, 2008, Giannopoulou et al., 2006). Comparisons between these reports and our data are hindered by methodological difference such as time of follow up, antibiotic type and/or regimen employed, inclusion of smokers, and GCF biomarkers assessed.

A recent paper suggested that the use of azithromycin could reduce the GCF levels of pro-inflammatory cytokines such as IL-1 β , IL-8 and TNF- α in periodontally healthy subjects (Ho et al. 2010). The authors concluded that this effect was a result of the anti-inflammatory properties of azithromycin. To our knowledge, neither metronidazole nor amoxicillin have anti-inflammatory effects. Therefore, the changes in GCF cytokines associated with the adjunctive use of antibiotics reported here should be interpreted as an indirect result of the antimicrobial effects of these drugs. In our previous report on the 3-month clinical and microbial outcomes of this trial, we demonstrated that the adjunctive use of systemic metronidazole and amoxicillin was associated with a greater reduction in the proportions of red and orange complex species, compared to the placebo group (Mestnik et al., 2010). The 6-month microbiological data confirm these preliminary findings (data not shown).

We could not demonstrate any statistically significant difference in the effect of the two different therapies on GCF cytokine content. However, we must emphasize that this study was not originally designed to detect difference between the clinical groups regarding the effects of the two therapies on GCF cytokine levels. Therefore, our findings should be interpreted with this limitation in mind.

In summary, our results provided additional support to the notion that GAgP is accompanied by a local imbalance in the levels of the pro-inflammatory cytokine IL-1 β and the anti-inflammatory cytokine IL-10. The data indicated that periodontal therapy partially reversed the ratio IL-1 β /IL-10 to levels found in periodontal health. Comparisons between the two therapies could not demonstrate significant differences. Finally, the reduction in GCF levels

of GM-CSF as a result of periodontal therapy indicates that this cytokine may play a role in the pathogenesis of GAgP.

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Clinical Relevance

Scientific rationale for study

Differences between cytokine profiles have been described between GCF samples taken from periodontal health and aggressive periodontitis. This investigation determined whether an intervention (periodontal therapy) diminished these differences.

Principal Findings

Periodontal therapy changed the cytokine profile of aggressive periodontitis GCF samples to one more similar to periodontal health. GCF GM-CSF also decreased after therapy, implicating this cytokine in the pathogenesis of GAgP. The two therapies did not differ in their effects on GCF cytokines.

Practical Implications

Better definition of the GCF cytokine profiles in GAgP could lead to biomarkers that would improve the diagnosis and monitoring of this condition.

Figure 1.

Plots of GCF cytokine levels (pg/ml) for each site sampled in the GAgP subjects at baseline and 6 months post-therapy. The green circles represent the baseline GCF levels and have been ordered in each panel from the site with the lowest GCF value to the highest value pre-therapy. The red and purple circles represent the 6 month GCF levels for each site. The red circles indicate increase, while the purple circles indicate a decrease after treatment in the site levels of GCF cytokines.

Figure 2.

Plots of mean GCF cytokine levels (pg/ml) at baseline and 6 months post-therapy in generalized aggressive periodontitis subjects in the placebo (white panels) and systemic antibiotic (grey panels) groups. The green circles represent the mean baseline GCF levels (measured at up to 14 sites per subject) and have been ordered in each panel from the subject with the lowest GCF value to the highest value pre-therapy. The red and purple circles represent the 6 month mean GCF data for each subject. The red circles indicate a mean increase, while the purple circles indicate a mean decrease in GCF cytokine levels after treatment.

Table 1

Mean ($\pm$ SD) demographic and clinical parameters at baseline and 6 months for the two treatment groups and for the control group

Parameter	SRP + placebo (n=12)	SRP + MET and AMX (n=12)	Control (n=25)
Age (years)	26 $\pm$ 3	27 $\pm$ 3	26 $\pm$ 3
% males	33	50	45
	Baseline	Baseline	Change
PD (mm)	3.9 $\pm$ 0.5 [†]	4.4 $\pm$ 0.6 [†]	2.3 $\pm$ 0.5
CAL (mm)	4.0 $\pm$ 0.5 [†]	4.7 $\pm$ 0.8 [†]	1.7 $\pm$ 0.4
% BOP	77 $\pm$ 18	77 $\pm$ 18	8 $\pm$ 9
NMT	2.3 $\pm$ 2.8	4.2 $\pm$ 4.0	0.5 $\pm$ 1.2
Pockets >4 mm	51 $\pm$ 15 (33)	60 $\pm$ 18 (42)	0
BOP+ve (%)	7 $\pm$ 9 (5)	3 $\pm$ 5 (3)	

^{†††} p<0.01, compared to baseline using Wilcoxon signed ranks test.

[†] p<0.05 between therapy groups using Mann-Whitney

^{§§} p<0.001,

[§] p<0.05 between groups using ANCOVA adjusting for baseline PD and AL

^{*} Mean values $\pm$ SD after ANCOVA adjustments

SRP - scaling and root planing; MET - metronidazole; AMX - amoxicillin

BOP - bleeding on probing

PD - pocket depth

CAL - clinical attachment level

NMT - number of missing teeth

Table 2

Mean ($\pm$ SD) GCF cytokine levels (pg/ml) for the Generalized Aggressive Periodontitis subjects at baseline and 6 months after therapy and for the control subjects

GCF Parameter	Generalized Aggressive Periodontitis (n=24)		Control (n = 25)
	Baseline	6-month	
GM-CSF (pg/ml)	4.2 $\pm$ 2.3	1.6 $\pm$ 1.0^{***}	2.2 $\pm$ 1.2
IFN- γ (pg/ml)	0.7 $\pm$ 1.1	0.4 $\pm$ 0.3	0.6 $\pm$ 1.2
IL-10 (pg/ml)	18.0 $\pm$ 9.0	22.6 $\pm$ 19.0	23.7 $\pm$ 19.9
IL-1 β (pg/ml)	19.3 $\pm$ 10.0	9.0 $\pm$ 13.9^{**}	7.0 $\pm$ 3.9
IL-2 (pg/ml)	1.8 $\pm$ 2.4	0.0 $\pm$ 0.0^{**}	0.7 $\pm$ 1.2
IL-6 (pg/ml)	3.2 $\pm$ 5.7	9.3 $\pm$ 16.0[*]	1.9 $\pm$ 3.9
TNF- α (pg/ml)	1.9 $\pm$ 1.8	3.5 $\pm$ 4.3	1.9 $\pm$ 1.4
IL-1 β /IL-10	2.5 $\pm$ 2.0	0.6 $\pm$ 0.6^{***}	0.5 $\pm$ 0.4

*
p<0.05,

**
p<0.01,

p<0.001 compared to baseline using Wilcoxon signed ranks test

GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; IFN, interferon; TNF, tumour necrosis factor; GCF, gingival crevicular fluid

Bold face indicates statistically significant differences from baseline to 6 months

Table 3Mean ($\pm$ SD) GCF cytokine levels (pg/ml) for the two clinical groups at baseline and 6 months after therapy

GCF Parameter	SRP + placebo (n=12)		SRP+ MET and AMX (n=12)	
	Baseline	6-month	Baseline	6-month
GM-CSF (pg/ml)	3.9 $\pm$ 2.9	1.7 $\pm$ 0.9 *	4.6 $\pm$ 1.5	1.6 $\pm$ 1.2 **
IFN- γ (pg/ml)	0.8 $\pm$ 1.3	0.5 $\pm$ 0.3	0.7 $\pm$ 0.9	0.4 $\pm$ 0.4
IL-10 (pg/ml)	19.2 $\pm$ 10.9	24.4 $\pm$ 18.6	16.9 $\pm$ 7.0	20.9 $\pm$ 20.0
IL-1 β (pg/ml)	18.8 $\pm$ 10.4	10.1 $\pm$ 13.0	19.7 $\pm$ 9.9	7.9 $\pm$ 15.2 *
IL-2 (pg/ml)	2.0 $\pm$ 2.7	0.0 $\pm$ 0.0 **	1.5 $\pm$ 2.1	0.0 $\pm$ 0.0 **
IL-6 (pg/ml)	4.4 $\pm$ 7.8	12.1 $\pm$ 21.6	2.0 $\pm$ 1.9	6.6 $\pm$ 7.4
TNF- α (pg/ml)	2.2 $\pm$ 2.4	3.7 $\pm$ 5.2	1.5 $\pm$ 0.9	3.3 $\pm$ 3.5
IL-1 β /IL-10	2.4 $\pm$ 1.6	0.6 $\pm$ 0.7 **	2.6 $\pm$ 2.4	0.6 $\pm$ 0.6 **

*
p<0.05 and**
p<0.01, compared to baseline using Wilcoxon signed ranks test

GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; IFN, interferon; TNF, tumour necrosis factor; GCF, gingival crevicular fluid; SRP, scaling and root planing; MET, metronidazole; AMX, amoxicillin

Bold face indicates statistically significant differences from baseline to 6 months