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Interleukin-1β levels in gingival crevicular fluid and serum under naturally occurring and experimentally induced gingivitis

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Abstract

Aims: To evaluate the interleukin-1β (IL-1β) levels in gingival crevicular fluid (GCF) and serum in either naturally occurring (N-O) or experimentally induced (E-I) plaque-associated gingivitis.

Material and Methods: Thirty-seven periodontally healthy subjects were evaluated in *real life* conditions (N-O gingivitis) as well as after 21 days of experimental gingivitis trial (E-I gingivitis). During the experimental gingivitis trial, in one maxillary quadrant (test quadrant), gingival inflammation was induced by oral hygiene abstention, while in the contralateral (control) quadrant, oral hygiene was routinely continued. IL-1β concentrations in N-O and E-I gingivitis were investigated for IL-1B^{+3954} and IL-1B^{-511} gene polymorphisms.

Results: (i) GCF IL-1β concentrations in E-I gingivitis were significantly higher compared with N-O gingivitis; (ii) an intra-individual correlation between GCF concentrations of IL-1β detected in N-O and E-I gingivitis was observed in control quadrants, but not in test quadrants; (iii) IL-1β concentration in GCF was associated with IL-1B^{+3954} genotype only at test quadrants; (iv) IL-1β was detectable in serum only at low levels in a limited number of subjects, without difference between gingivitis conditions.

Conclusions: Aspects of the bacterial challenge to the gingival tissues, such as the amount of plaque deposits and plaque accumulation rate, appear to affect the IL-1β levels in GCF in subjects with a specific IL-1B genotype.

Key words: experimental gingivitis; gingival inflammation; interleukin-1β

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During the past years, we developed and validated a human experimental gingivitis model and used it to identify some factors affecting the individual susceptibility to plaque-induced gingival inflammation (Trombelli et al. 2004a, b, c, 2005, 2006a, b, 2008, Scapoli et al. 2005, 2007). The reported significant differences in gingival inflammatory response under quantitatively and/or qualitatively almost identical plaque accumulation (Abbas et al. 1986, Lie et al. 1995, Trombelli et al. 2004a, b, c) suggest that the gingival response to bacterial challenge may be an individual trait (Abbas et al. 1986, Tatakis & Trombelli 2004), dependent on host-related factors, possibly genetic in origin (Tatakis & Trombelli 2004, Scapoli et al. 2005, 2007). A series of studies were, therefore, implemented and designed to test whether and to what extent the individual susceptibility to plaque-induced gingivitis vary depending on host-derived systemic factors, whether genetic or environmental.

The gingival inflammatory response to dental biofilm is partly mediated and regulated by proinflammatory cytokines, among which interleukin-1β (IL-1β) has been recognized to play a pivotal role (Barksby et al. 2007). Inflamed gingival tissues are characterized by an increased presence of IL-1β-secreting cells, such

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as macrophages and neutrophils (Lo et al. 1999), and the tissue content in IL-1β positively correlates with the extent of inflammatory cell infiltrate and the clinical severity of gingival inflammation (Liu et al. 1996, Hou et al. 2003). In this respect, it was demonstrated previously that gingival tissue specimens from sites with moderate to severe gingival inflammation exhibit higher concentrations of IL-1β compared with non-inflamed gingival sites (Ejeil et al. 2003).

When IL-1β levels were investigated under naturally occurring (N-O) plaque-induced gingival inflammatory conditions, patients with gingivitis showed significantly higher total amounts of IL-1β in the gingival crevicular fluid (GCF) compared with periodontally healthy individuals without clinical signs of gingival inflammation (Yücel et al. 2008). Moreover, Fitzsimmons et al. (2009) reported a significant correlation between the level of IL-1β in GCF and clinical indexes to define gingival inflammation. Consistently, the IL-1β concentration in the GCF has also been associated with the clinical severity of gingival inflammation in the experimental gingivitis model. After 3 days of neglected oral hygiene, an increased IL-1β concentration in the GCF was observed that preceded any clinical sign of gingival inflammation (Zhang et al. 2002). The abstention from mechanical plaque control for longer time intervals (18–28 days) induced an increase in IL-1β levels in GCF, which was associated with a clinically evident gingival inflammatory response (Deinzer et al. 1999, 2007, Gonzáles et al. 2001, Waschul et al. 2003, Schierano et al. 2008). Overall, these findings show that the IL-1β concentration in GCF can be seen as a reliable marker associated with the presence and severity of plaque-induced gingival inflammation. However, a high inter-individual variability of IL-1β concentration has been reported in GCF of patients showing gingival inflammation (Faizuddin et al. 2003, Bergmann & Deinzer 2008, Yücel et al. 2008), suggesting that IL-1β production elicited by the bacterial challenge may be under genetic regulation.

In a previous study, we evaluated the association between IL-1 gene variability and the clinical parameters of gingivitis. When two subgroups of subjects, who, respectively, exhibited high and low gingival inflammatory response to similar rates and levels of plaque accumulation, were compared for the frequency of IL-1B gene polymorphisms, a significant inter-group difference in genotype distribution was observed for IL-$1B^{-511}$ polymorphism (Scapoli et al. 2005). At present, however, there is still insufficient scientific evidence to clearly define the relationship between IL-1B genotype, GCF levels of IL-1β and patient susceptibility to gingival inflammation (Greenstein & Hart 2002).

Recently, we advanced the hypothesis that our experimental model could be used to identify *a priori* (i.e. under N-O gingival inflammation) subjects who will develop a low or high gingival inflammatory response under experimental conditions (i.e. at completion of a 21-day experimental gingivitis trial). In this respect, we found that subjects who had showed a consistently higher and lower severity of plaque-induced gingival inflammation following two consequent experimental gingivitis trials also showed a consistently higher and lower gingival inflammation when observed in their ''natural state'' (Trombelli et al. 2008). Therefore, in the present study, we adopted our model to evaluate the IL-1β levels in GCF and serum in either N-O or experimentally induced (E-I) plaque-associated gingivitis. The influence of gene polymorphisms on the phenotypic expression of serum as well as GCF IL-1β concentration was also investigated.

Material and Methods

Experimental design and study population

The study protocol was approved by the Research Ethics Committee, University of Ferrara, and all participants provided written informed consent. After a first randomized split-mouth localized experimental gingivitis clinical trial (first trial), conducted from October 2000 to November 2001, two sub-populations of periodontally healthy individuals were identified, respectively, defined as high responders (HR, $n = 24$) and low responders (LR, $n = 24$), and characterized by significantly different severity of gingivitis to similar amounts of plaque deposits (Trombelli et al. 2004c). On January 2002, we recalled all HR and LR individuals to verify their availability and eligibility for a second trial (repeat trial) (Trombelli et al. 2008). Volunteers underwent the repeat trial between April and November 2002. Therefore, we could discriminate two subsets of individuals who showed a consistently higher (HR/HS, $n = 10$) and lower (LR/LS, $n = 10$) inflammatory response, as clinically assessed, to similar amount of plaque accumulation after both first and repeat experimental gingivitis trials (Trombelli et al. 2008).

In each subject, one maxillary quadrant was randomly assigned as ''test'', the contralateral quadrant served as ''control''. Two weeks before the repeat experimental gingivitis trial, subjects underwent a first evaluation, including the assessment of clinical and immunological parameters as described below. Plaque accumulation, gingival inflammation and qualitative/quantitative assessment of GCF were recorded in test and control quadrants when the subject was in *naïve* (real life) conditions. These conditions were regarded as N-O gingivitis. At first evaluation, after professional scaling and polishing a medium toothbrush (Elmex Inter X, GABA International AG, Münchenstein, Switzerland), unwaxed floss (Elmex, GABA International AG) and standard toothpaste (Aronal, GABA International AG), along with oral hygiene instructions, were provided. Toothpaste was provided in masked tubes. Subjects were taught the modified Bass technique or, if their hygiene regimen was judged sufficient, only few individual instructions were given on how to improve their performance (Trombelli et al. 2004c, 2008). At 14 days after the first evaluation, the volunteers underwent a 21-day experimental gingivitis trial. In test quadrants, experimental gingivitis was induced by oral hygiene abstention; in control quadrants, oral hygiene was continued to ensure proper plaque control (Trombelli et al. 2004c, 2008). After 21 days of experimental gingivitis, all subjects were re-evaluated for clinical and immunological parameters. These conditions were regarded as E-I gingivitis. Therefore, each subject was evaluated in two different conditions of plaque accumulation and related gingival inflammation at both test and control quadrants: N-O and E-I gingivitis.

Clinical parameters

At first evaluation and after 21 days of experimental gingivitis, the following parameters were assessed at the buccal and the mesiobuccal aspects of the maxillary lateral incisors, first bicuspids

and first molars, in the order listed: gingival index (GI), plaque index (PlI) and angulated bleeding score (AngBS). All parameters were recorded by two trained and calibrated examiners with good to excellent intra- and inter-examiner agreement (Trombelli et al. 2004c, 2008).

IL-1β in serum and GCF

At first evaluation, 20 ml of venous blood was collected from each patient in vacutainer tubes. The serum was isolated by centrifugation after clotting and stored at -80°C. The concentration of IL-1β in the serum (sIL-1β) was quantified by a high-sensitivity enzyme-linked immunosorbent assay (s-Es; Quantikine HS, R&D System, Minneapolis, MI, USA) using commercially available OptEIA sets for human IL-1β (PharMingen, San Diego, CA, USA), according to the manufacturer's instructions.

At first evaluation and after 21 days of experimental gingivitis, sampling of GCF for IL-1β concentration (cIL-1β) was performed at the buccal and the mesiobuccal aspect of the maxillary lateral incisors, first bicuspids and first molars at test and control quadrants. GCF was collected after GI and PlI assessment. After isolation of the area (cotton roll or gauze) and removal of supragingival plaque with a curette or scaler, GCF was collected on paper strips (Perio-paper®; IDE Interstate, Amityville, NY, USA), as described previously (Fransson et al. 1999). The strips were placed in vials containing a transport medium (1 ml of PBS and bovine serum albumin) and stored at -70°C until GCF extraction. To perform GCF extraction, the vials were sonicated for 5 min. After centrifugation, the extracted supernatants were aliquoted in five eppendorf vials (each containing 200 μl) and stored at -80°C until analysis. GCF samples and serum were analysed for IL-1β using commercially available enzyme-linked immunosorbent assays (ELISA hIL-1β; Bio Source International, Camarillo, CA, USA). Analyses were performed according to the manufacturer's instructions. All ELISA determinations were performed in duplicate. Results were calculated using the standard curves created in each assay. Concentrations of the cytokine were corrected for GCF volume and defined as picogram per millilitre.

Analysis of IL-1B polymorphisms

Methods for the analysis of IL-1B polymorphisms from peripheral blood were reported previously (Scapoli et al. 2005). The following IL-1B polymorphisms were analysed using polymerase chain reaction (PCR):

- IL-1B^{+3954}: The PCR targeted the 194 bp region of IL-1B. Genotypes of the IL-1B^{+3954} restriction fragment length polymorphism (RFLP) (allele 1: C; allele 2: T) were determined with Taq1 (GeneRuler DNA Ladder Plus; Promega, Milan, Italy). Two fragments of 86 and 108 bp were observed in subjects homozygous for allele 1 and a single fragment of 194 bp in subjects homozygous for allele 2.
- IL-1B^{-511}: The 304 bp region on the promoter of IL-1B was amplified and genotypes of the IL-1B^{-511} RFLP (allele 1: C; allele 2: T) were determined with AvaI (Amersham Biosciences, Cologno Monzese, Italy). Two fragments of 194 and 114 bp were observed in subjects homozygous for allele 1 and a single fragment of 304 bp in subjects homozygous for allele 2.

Statistical analysis

The subject was the statistical unit. For each parameter, recordings from the six selected sites (three selected teeth; two sites per tooth) per quadrant were averaged to obtain the subject mean value for each test and control quadrant. Therefore, for each parameter at each observational period, the subject was represented by a single test and a single control value.

Kolmogorov–Smirnov goodness-of-fit tests were computed for each variable to assess whether the variables were normally distributed. Data were expressed by either mean ± standard deviation (SD) for parametric variables (PlI, GI, GI/PlI ratio) or median and inter-quartile range (IR) for non-parametric variables (AngBS, cIL-1β, sIL-1β). Comparisons were performed with Student's *t*-test for paired observations and Wilcoxon test for parametric and non-parametric variables, respectively. To test the effect of different groups or genotypes on clinical and immunological parameters, Kruskal–Wallis test was used. Multiple post hoc comparisons of the average ranks for each pair of groups, as well as post hoc probabilities (corrected for the number of comparisons) for a two-sided test of significance, have been computed when necessary. Pearson and Spearman correlation tests were used to assess the strength of association between parametric and non-parametric variables, respectively. The level of significance was set at 5%.

Results

Study population

Thirty-seven (20 males/17 females; mean age: 23.7 ± 1.8 years) underwent the first evaluation and completed the experimental gingivitis trial. In one subject, the analysis of IL-1β in GCF was not possible due to technical reasons. Therefore, the statistical analysis was performed on 36 subjects.

Plaque accumulation and gingival inflammation in N-O and E-I gingivitis

The values of PlI, GI and AngBS in N-O and E-I gingivitis in test and control quadrants are shown in Table 1. In test quadrants, more severe supragingival plaque accumulation (PlI, $p<0.0001$) and gingival inflammation (GI and AngBS, $p<0.0001$) were observed in E-I gingivitis compared with N-O gingivitis. In contrast, significantly lower PlI ($p<0.01$) and GI ($p<0.001$) were observed in E-I gingivitis compared with N-O gingivitis at control quadrants. In N-O gingivitis, no significant differences in PlI, GI and AngBS were observed between test and control quadrants. In E-I gingivitis, PlI, GI and AngBS were significantly higher in the test quadrant when compared with the control quadrant ($p<0.0001$).

cIL-1β and sIL-1β in N-O and E-I gingivitis

The values of sIL-1β as well as cIL-1β in test and control quadrants as assessed in N-O and E-I gingivitis are shown in Table 1. A significantly higher cIL-1β was observed in E-I gingivitis compared with N-O gingivitis at both test ($p<0.0001$) and control ($p<0.001$) quadrants. In E-I gingivitis, cIL-1β was significantly higher in test compared with control quadrants ($p<0.0001$). A statistically significant positive correlation between cIL-1β in N-O and E-I gingivitis was found in control quadrants (Spear-

Table 1. Plaque index (PlI), gingival index (GI) and angulated bleeding score (AngBS), concentration of interleukin-1β (IL-1β) in gingival crevicular fluid (cIL-1β) in test and control quadrants as well as concentration of IL-1β in serum (sIL-1β) as assessed in naturally occurring (N-O) and experimentally induced (E-I) gingivitis

Experimental parameter	Observation interval	Control quadrant		Test quadrant		*p*-value
		N	mean ± SD or median (IR)	*N*	mean ± SD or median (IR)	
PlI	N-O gingivitis	36	0.6 ± 0.3	36	0.6 ± 0.3	0.191
	E-I gingivitis	36	0.5 ± 0.3	36	1.8 ± 0.4	<0.0001
			$p<0.01$		$p<0.0001$	
GI	N-O gingivitis	36	0.4 ± 0.3	36	0.4 ± 0.2	0.618
	E-I gingivitis	36	0.2 ± 0.2	36	0.9 ± 0.2	<0.0001
			$p<0.001$		$p<0.0001$	
AngBS	N-O gingivitis	36	0 (0–0.2)	36	0 (0–0)	0.091
	E-I gingivitis	36	0 (0–0)	36	0.3 (0.2–0.5)	<0.0001
			Not significant		$p<0.0001$	
cIL-1β (pg/ml)	N-O gingivitis	36	0 (0–20.9)	36	9.8 (0–19.5)	0.264
	E-I gingivitis	36	18.1 (7.6–22.5)	36	25.2 (22.5–31.1)	<0.0001
			$p<0.001$		$p<0.0001$	

Experimental parameter	Observation interval	*N*	Mean ± SD or median (IR)
sIL-1β (pg/ml)	N-O gingivitis	36	0 (0–0)
			0 (0–0)
	E-I gingivitis	36	
			$p = 0.938$

Values are expressed as mean ± standard deviation (SD) or median and inter-quartile range for parametric and non-parametric variables, respectively.

man = 0.34, $t_{N-2} = 2.12$, $p<0.05$), but not in test quadrants (Fig. 1).

sIL-1β was detectable in six subjects in N-O gingivitis and seven subjects in E-I gingivitis, and remained unchanged throughout the study ($p>0.05$).

IL-1β levels in HR/HS and LR/LS

When the two subsets of HR/HS and LR/LS individuals (Trombelli et al. 2008) were compared for IL-1β levels in serum and GCF, no significant differences could be detected at either test and control quadrants under N-O and E-I conditions.

Association between IL-1B polymorphisms and IL-1β concentration

No significant association was found between IL-1B^{+3954} genotype and IL-1β concentrations in N-O gingivitis. In E-I gingivitis, IL-1B^{+3954} genotype was significantly associated with cIL-1β in test quadrant ($p<0.01$), but not with cIL-1β in the control quadrant. The values of test quadrant cIL-1β in E-I gingivitis in the study population subgrouped for the different IL-1B^{+3954} alleles are shown in Table 2. TT homozygotes showed a significantly higher cIL-1β compared with CC homozygotes ($p = 0.007$) and a borderline difference compared with heterozygotes ($p = 0.06$). No statistically significant differences in PlI were found between different IL-1B^{+3954} genotypes at test quadrants in either N-O ($p = 0.99$, data not shown) and E-I gingivitis ($p = 0.25$, Table 2).

No statistically significant association was found between IL-1B^{-511} genotype and cIL-1β in either N-O or E-I gingivitis. sIL-1β levels were not associated with either IL-1B^{-511} or IL-1B^{+3954} genotypes.

Discussion

The present study was performed to evaluate the IL-1β levels in serum and GCF in subjects presenting plaque-induced gingival inflammation. Each subject was evaluated in terms of plaque accumulation and gingival inflammation, GCF and serum concentration of IL-1β in *real life* conditions (N-O gingivitis) as well as after 21 days of experimental gingivitis trial (E-I gingivitis). In both N-O and E-I gingivitis, one maxillary quadrant was randomly assigned as ''test'', the contralateral quadrant served as ''control''. During the experimental gingivitis trial, in test quadrants, gingival inflammation was induced by oral hygiene abstention; in control quadrants, oral hygiene was continued to ensure proper plaque control. GCF and serum concentrations of IL-1β in N-O and E-I gingivitis were also investigated for different gene polymorphisms. The results indicated that (i) GCF IL-1β concentrations in E-I gingivitis were significantly higher compared with N-O gingivitis; (ii) an intra-individual correlation between GCF concentrations of IL1β detected in N-O and E-I gingivitis was observed in control quadrants, but not in test quadrants; (iii) IL-1β concentration in GCF was associated with IL-1β^{+3954} genotype only at quadrants where gingival inflammation had been elicited by E-I plaque accumulation; (iv) IL-1β was detectable in serum only at low levels in a limited number of subjects, without difference between gingivitis conditions.

In our study, IL-1β was detectable in serum only at low levels in a small number of subjects, without difference between gingivitis conditions. This may be partly due to the limited severity of the plaque-induced gingival inflammation, which characterized our study population at both N-O and E-I gingivitis. Consistently, previous studies have shown that IL-1β was found in the serum at extremely low concentrations in periodontally healthy (Mengel et al. 2002) and gingivitis (Orozco et al. 2006)

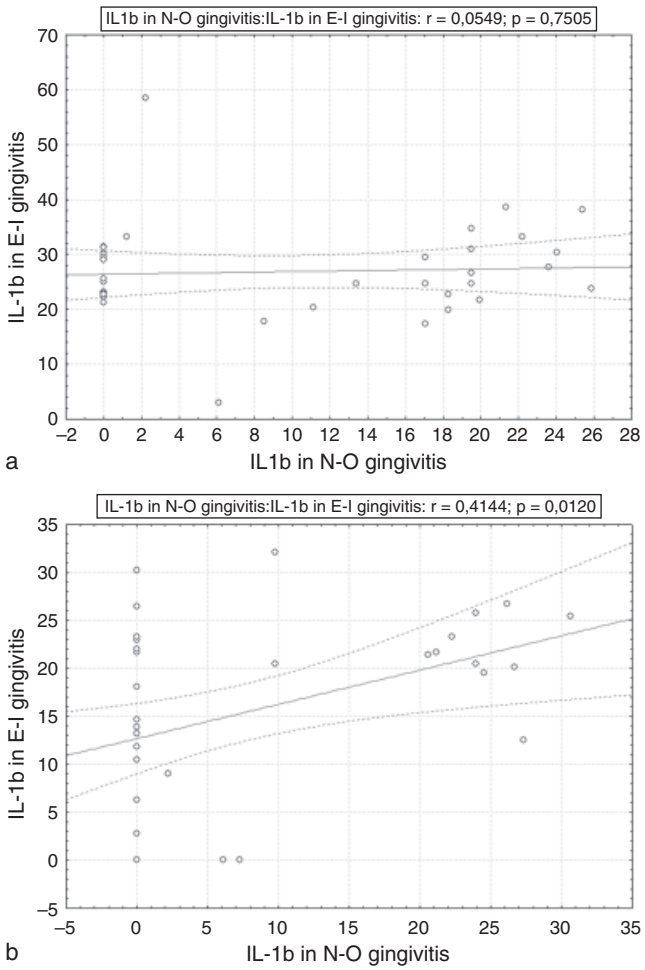

Fig. 1. Distribution of subjects (dots) according to the concentration (pg/ml) of interleukin-1β (IL-1β) in gingival crevicular fluid as detected in naturally occurring (N-O) gingivitis and experimentally induced (E-I) gingivitis in test (a) and control quadrant (b). Continuous line: linear correlation; dotted line: 95% confidence interval.

patients, and the systemic concentrations of IL-1β are positively correlated with the degree of gingival inflammation (Liu et al. 1996, Ejeil et al. 2003, Hou et al. 2003, Orozco et al. 2006, Fitzsimmons et al. 2009).

A higher IL-1β concentration in GCF was found in E-I compared with N-O gingivitis at both test and control quadrants. In test quadrants, an increased expression of GCF IL-1β was associated with an increased amount of plaque accumulation (PlI) paralleled by an increased severity of gingival inflammation (GI, AngBS). Interestingly, a significantly higher concentration of IL-1β was detected in E-I compared with N-O gingivitis even at control quadrants despite significantly lower PlI and GI. These observations are partly consistent with those reported by previous studies where subjects exposed to experimental gingivitis showed significantly more plaque accumulation and higher IL-1β concentrations than subjects with spontaneous gingivitis (Deinzer et al. 2007). In our study design, N-O gingivitis was completely reversed at experimental sites by means of professional supra- and sub-gingival plaque removal under optimal conditions of self-performed plaque control (Trombelli et al. 2004c, 2008). At the end of the experimental gingivitis trial, gross supra-gingival plaque deposits were particularly evident in the test quadrant, PlI being 3-fold higher in E-I compared with N-O gingivitis (Table 1). Therefore, it may be speculated that the higher IL-1β concentrations observed in E-I gingivitis may reflect the boost in the microbial challenge to the gingival tissues associated with the experimental conditions of plaque accumulation. However, an increase in plaque deposits from the time of pre-trial professional plaque removal to the completion of the experimental gingivitis trial is also conceivable for control quadrants. This bacterial challenge determined an acute, although more limited, inflammatory response of the gingival tissues associated with increased GCF levels of IL-1β. While IL-1β in GCF was detected in 22 and 15 subjects at test and control quadrants respectively in N-O, the cytokine detection involved 36 and 31 subjects at test and control quadrants respectively in E-I (Fig. 1a, b). Consistently, previous experimental gingivitis studies have demonstrated that IL-1β release rates increase in GCF at an early phase as a consequence of a limited amount of plaque accumulation, even before any clinical signs of inflammation appear (Zhang et al. 2002).

Although we do not have data regarding plaque and gingivitis of the volunteers before the first evaluation, it may be reasonable to assume that the supragingival plaque deposits observed in N-O gingivitis may reflect the oral hygiene standard of the subject. This persistent microbial challenge may have resulted in a steady, ''chronic'' inflammatory response of the gingival tissues, which appears characterized by a lower expression of GCF IL-1β compared with E-I gingivitis. These findings are consistent with previous experimental gingivitis studies where significant time-related fluctuations in GCF IL-1β concentrations were observed in experimental (acute) gingivitis conditions but not in a persistent (chronic) gingivitis status (Deinzer et al. 2007).

A positive correlation was found between individual GCF concentrations of IL-1β under N-O and E-I inflammatory conditions in control quadrants, but not in test quadrants. In other words, at sites with limited amount of supragingival plaque accumulation and gingival inflammation, a consistently high or low GCF IL-1β concentration was observed in both N-O and E-I gingivitis. However, at sites where the bacterial challenge and related gingival inflammation substantially increased due to neglected oral hygiene, higher concentrations of IL-1β were detected independently from the GCF IL-1β concentrations as assessed in N-O gingivitis. Taken together with the observed differences in GCF concentrations of IL-1β detected in E-I compared with N-O gingivitis, these findings appear to suggest that

Table 2. Concentration (pg/ml) of interleukin-1β (IL-1β) in gingival crevicular fluid (cIL-1β) and plaque index (PlI) as assessed in test quadrant after experimentally induced gingivitis for IL-1B^{+3954} genotypes

	IL-1B^{+3954} genotypes		
	CC ($n = 15$)	CT ($n = 17$)	TT ($n = 4$)
cIL-1β	23.0 (21.2–29.0)	26.7 (23.2–31.0)	35.6 (32.2–48.3)*
PlI	1.9 ± 0.4	1.7 ± 0.3	1.7 ± 0.2

cIL-1β and PlI are expressed as median (inter-quartile range) and mean ± standard deviation, respectively.

*TT homozygotes showed a significantly higher IL-1β concentration compared with CC homozygotes ($p = 0.007$) and a borderline difference compared with heterozygotes ($p = 0.06$).

aspects of the bacterial challenge to the gingival tissues, such as the amount of plaque deposits and plaque accumulation rate, affect the GCF levels of IL-1β.

In a previous study, we could discriminate two subsets of individuals who showed a consistently higher (i.e. HR/HS) and lower (i.e. LR/LS) inflammatory response, as clinically assessed, to similar amount of plaque accumulation after both first and repeat experimental gingivitis trials (Trombelli et al. 2008). Interestingly, the HR/HS individuals presented a significantly higher test quadrant GI compared with LR/LS individuals under both N-O and E-I gingivitis conditions despite similar plaque deposits. Moreover, HR/HS individuals showed a GI/PlI ratio significantly higher than LR/LS individuals under N-O gingivitis, suggesting that it might be possible to select a priori, on the basis of presenting level of gingival inflammation, subjects with different degrees of gingival inflammatory response to de novo plaque accumulation (Trombelli et al. 2008). Apparently, these differences in gingival response to plaque as clinically observed in these two subsets are not reflected by significant differences in the levels of sIL-1β under either ''natural'' or ''experimental'' gingivitis conditions.

In the present study, we investigated the association between the expression of IL-1β in GCF and serum with two polymorphisms for the IL-1B gene within the promoter region (at -511) and in exon V (at $+3954$). Despite a small sample size, our results confirm data from previous studies where IL-1B^{+3954} genotype has been related to IL-1β production (Pociot et al. 1992, Di Giovine et al. 1995). In particular, TT genotype showed a significantly higher GCF IL-1β level compared with CC genotype and a borderline difference compared with CT genotype. These differences in cIL-1β among genotypes could not be explained by differences in the amount of plaque accumulation (Table 2). It is noteworthy that IL-1B^{+3954} genotype was found to be associated with cIL-1β only at sites where an increased bacterial challenge to the gingival tissues was provided by 21 days of undisturbed plaque accumulation (test quadrants). These findings are consistent with experimental data showing that IL-1 secretion by cultured explants of chorioamnion carrying the IL-1B*2 polymorphism gene was increased dramatically upon stimulation with LPS in comparison with those carrying the more common allele, and that such an increase was dose dependent (Hernandez-Guerrero et al. 2003). It may be speculated that the quantitative (Löe et al. 1965) and qualitative (Theilade et al. 1966) changes in plaque deposits, as induced by undisturbed plaque accumulation in the experimental gingivitis model, may represent an essential condition for the genetic modulation in GCF IL-1β production to become manifest. In this context, it has also been demonstrated that IL-1B^{+3954} genotype-positive subjects are more prone to harbour higher counts of species in red and orange complexes compared with genotype negative subjects (Socransky et al. 2000).

In conclusion, our results indicate that in N-O gingivitis, plaque deposits determine an inflammatory condition of the gingival tissues, which is associated with the IL-1β expression in GCF. An imbalance in the amount (and, maybe, composition) of the bacterial challenge triggers an increased production of the pro-inflammatory cytokine, which is particularly pronounced in subjects with a specific IL-1B genotype. These findings appear to be of clinical relevance due to the potential role of IL-1β in periodontal disease progression and as an indicator of active inflammation (Lo et al. 1999, Graves & Cochran 2003). Recent studies on the characterization of host response assessed in periodontal lesions of chronic periodontitis (ChP) patients have demonstrated higher GCF levels of pro-inflammatory cytokines, including IL-1β, in active sites (i.e. sites experiencing an acute destruction of periodontal attachment) compared with inactive sites (Silva et al. 2008). In this respect, it has been reported that IL-1B^{+3954} CT and TT genotypes were associated with higher IL-1β mRNA expression in periodontal tissues in ChP patients, but not in gingivitis patients. In addition, a summative, positive effect of IL-1B^{+3954} genotype and the red complex periodontopathogens was found on the modulation of IL-1β in periodontal tissues (Ferreira et al. 2008). Therefore, further studies are needed to better understand the role of IL-1β not only as a marker of plaque-induced gingival inflammation but also as a potential pivot in the complex host–parasite interaction leading to the progression from gingivitis to destructive periodontitis.

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Clinical Relevance

Scientific rationale for the study: The modulation of IL-1β production in serum and GCF either under N-O and E-I gingivitis has not been completely clarified yet.
Principal findings: GCF IL-1β concentration was significantly higher in E-I gingivitis than N-O gingivitis. In E-I gingivitis, IL-1B^{+3954} genotype influenced IL-1β concentration in GCF only at sites where gingival inflammation was elicited by gross plaque accumulation.
Practical implications: Aspects of the bacterial challenge to the gingival tissues, such as plaque deposits and plaque accumulation rate, appear to affect the IL-1β levels in GCF in subjects with a specific IL-1B genotype.