The Significance of Gingival Crevicular Fluid and IL-1β, TNF-α and PGE_2 in Periodontal Disease. A Literature Review

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Purpose: To review the literature regarding cytokines and PGE_2 levels in gingival crevicular fluid (GCF) in healthy and diseased periodontium.

Materials and Methods: A Medline search ending at March 2004 was performed, in the English literature, regarding IL-1β, TNF-α and PGE_2 in the GCF, and the data was reviewed systematically.

Results: Low levels of these markers were reported in healthy periodontium. Significant increase in the amount of IL-1β and PGE_2 was noted in gingivitis and periodontitis. Most of the studies have shown that there is a marked decrease in IL-1β and PGE_2 levels of periodontal disease patients after therapy, while inconsistent results were found for TNF-α.

Conclusions: The IL-1β, TNF-α and PGE_2 levels may be indicative of periodontal status, and amount rather than concentration being more reliable in reflecting the real status of the periodontium.

Key words: cytokine, gingival crevicular fluid, periodontal disease, IL-1β, TNF-α, PGE_2

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INTRODUCTION

Bacteria are essential for the initiation and progression of periodontal disease; however, neither bacterial quantity nor specific bacterial species have been sufficient to explain the differences in disease severity between individuals. The available data suggests that several factors that amplify the inflammatory process make people more susceptible to periodontal disease than others. Patient susceptibility is of utmost importance to the outcome of periodontal disease; while periodontal bacteria are the primary etiological agents, the host immune response to these bacteria is of fundamental importance (Seymour, 1991).

The host response can be broadly classified into 3 categories: the humoral immune response (e.g. antibody production), cellular immune response (e.g. cytokines production), and acute inflammatory response (e.g. PGE_2).

Given the B-cell nature of the periodontitis lesion, it has been proposed that individuals susceptible to periodontal disease may have a Th2 response, whereas resistance to periodontal disease may be related to a Th1 cytokine profile (Gemmell and Seymour, 1994). Cytokines are soluble proteins or glycoproteins released from cells involved in immunoinflammatory processes and are modulating the activity of the immune and/or other cells (Bendtzen, 1989; Bendtzen, 1991). They seem vital in the immunopathology of an ever-increasing number of diseases, while the production of 'appropriate' cytokines is essential for the development of protective immunity. If 'inappropriate' cytokines are elicited, destructive or progressive disease might result (Figueredo et al, 1999).

The list of identified interleukins grows continuously with the total number of individual activities now at 22. It has been shown by many investigators that interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumour necrosis factor-α(TNF-α) can be detected in gingival crevicular fluid (GCF) in periodontal disease (Rossomando et al, 1990; Geivelis et al, 1993; Payne et al, 1993) and that the cytokine levels in GCF are closely associated with the severity of gingival inflammation and/or periodontal tissue destruction (Masada et al, 1990; Stashenko et al, 1991). It has been shown that IL-1α, IL-1 β, IL-6, IL-8, and TNF-α can be detected in the gingival crevicular fluid (GCF). Higher levels of these mediators in the GCF are detected in periodontal disease (Rossomando et al, 1990; Geivelis et al, 1993; Payne et al, 1993); cytokine levels being closely associated with the severity of gingival inflammation and/or periodontal tissue destruction (Masada et al, 1990; Stashenko at al, 1991).

IL-8, that is produced by a wide variety of cells (polymorphonuclear leukocytes, monocytes, macrophages and fibroblasts), plays a key role in the accumulation of leukocytes at the sites of inflammation (Bickel, 1993) and its level is known to increase in the GCF of inflamed as compared to healthy sites (Tsai et al, 1995). The pivotal role of IL-1β and TNF-α in the pathogenic mechanisms of periodontitis has been established by Page (1999); and cause-and-effect relationship between cytokines and periodontal tissue loss has been further confirmed (Assuma et al, 1998; Graves et al, 1998; Delima et al, 2001). IL-1β has been shown to stimulate bone resorption and to have inhibitory effects on bone formation (Tatakis, 1993). In addition, it is suspected to be involved in other osseous pathologies, as well as periodontitis (Nguyen et al, 1991).

A notable property of IL-1β in the pathological process of periodontitis is the stimulation of matrix metalloproteinases (MMPs) production (collagenases, gelatinases, stromelysins). These MMPs are able to degrade the extracellular matrix macromolecules (Van Der Zee et al, 1997), thus causing tissue destruction. It is now generally agreed that IL-1β is the major mediator of tissue destruction in periodontal disease (Page et al, 2000).

Periodontal ligament and gingival fibroblasts challenged with IL-1β in vitro release PGE_2 in a dose dependent manner (Richards and Rutherford, 1988) and secrete collagenases and matrix metalloproteinases (Birkedal-Hansen, 1993). IL-1β up-regulates cyclooxygenase-2 (COX-2) mRNA and may increase the stability of COX-2 in vivo (Ristimaki et al, 1994). The increase in tissue COX concentration by IL-1β may, in part, explain the higher levels of the arachadonic acid metabolite PGE_2 consistently observed in the GCF of patients with periodontitis.

TNF-α, a trimeric protein with a molecular weight of approximately 17 kDa/monomer (Vilcek and Lee, 1991), is secreted by monocytes and macrophages. TNF-α is a potent immunologic mediator with proinflammatory properties (Birkedal-Hansen, 1993). It also induces the secretion of collagenase by fibroblasts (Dayer, 1985), the resorption of cartilage (Saklatvala, 1986) and bone (Bertolini et al, 1986), and has been implicated in the destruction of periodontal tissue during periodontal disease (Meikle et al, 1986). Lipopolysaccharide (LPS) obtained from periodontal Gram-negative bacteria can initiate the production of TNF-α by peripheral blood monocytes (Lindemann et al, 1988), thus leading to enhanced tissue destruction.

PGE_2 is an arachidonic acid metabolite that has potent pro-inflammatory and immunomodulatory effects. There is considerable evidence correlating GCF PGE_2 levels with the severity of clinical disease (for review see Offenbacher et al, 1993a) and, more importantly, with bursts of active disease. In fact, PGE_2 has been implicated as a key proinflammatory mediator in periodontal disease (Offenbacher et al, 1993b; Lamster, 1997; Heasman et al, 1998; Tsai et al, 1998; Offenbacher et al, 1998; Lamster and Grbic, 2000). Macrophages are believed to be the major source of PGE_2 in GCF.

The aim of the present paper is to review the available English literature regarding the GCF and IL1-β, TNF-α and PGE_2 profiles in healthy and diseased periodontium.

GINGIVAL CREVICULAR FLUID (GCF)

GCF is an inflammatory exudate of the periodontal tissues. The fluid component is derived primarily from serum, while fluid constituents originate from structural cells in the connective tissue, epithelium and subgingival bacteria (Lamster and Grbic, 1995). GCF amount is indicative only of the degree of gingivitis (Fine and Mandel, 1986), while not necessarily of periodontal inflammation (Shapiro et al, 1979). The immune system is active in the GCF and includes the humoral, cellular and the acute inflammatory responses (Lamster, 1992). Since host response is a critical determinant in periodontal disease pathogenesis, the measure of inflammatory mediator levels in the GCF has been used to evaluate risk for a site to loose attachment and/or alveolar bone, or

the risk for an individual to develop periodontal disease (Champagne et al, 2003).

GCF concentration of varying pro-inflammatory cytokines is well documented. Recently, more studies are using total mediator content, rather than concentration, to report their results. The former has the advantage of eliminating the requirement to measure GCF volume, which is typically small (<1 μl) and thus susceptible to disproportionately large errors (Lamster et al, 1988; Adonogianaki et al, 1996). Due to the problem associated with measuring the extremely small quantities of GCF available from healthy sites, the levels of biochemical compounds have been presented by several authors as total amounts per 30-second sample, as an alternative to concentrations (Williams et al, 1989; Offenbacher et al, 1990; Offenbacher et al, 1992).

In order to standardize measurements, if total mediator content is to be used as an outcome variable, the sampling procedure should be standardized by sampling at a pre-determined time frame (usually 30 s). It is important to notice the effect of 'collection time'. A too short time (<20 s) may result in insufficient volume, and on the other hand, a long time (e.g. 1 minute) may result in an over-saturated paper strip.

The dilemma of concentration versus total amount was pointed out by Tsai et al (1995), who showed that following initial periodontal treatment GCF IL-1β concentration either remained unchanged or increased, while the total amounts decreased. The findings that IL-1β concentrations were negatively correlated with the GCF volumes, while total amounts were positively correlated with GCF volumes suggest that the reduction of GCF volumes as the result of periodontal treatment may be one of the factors responsible for the reduction of cytokine levels. Therefore, it is important to standardize sampling time and techniques.

LEVELS OF IL-1β, TNF-α AND PGE2 IN THE GCF

Healthy Periodontium

In clinically healthy gingival tissues, inflammatory cytokines are present in low quantities, suggesting cytokines are also prominent actors of normal tissue homeostasis (Okada and Murakami, 1998). It is now known, that the release of cytokines is genetically determined, and there is an association between a specific composite genotype of the IL-1β gene cluster and periodontal disease severity (Kornman et al, 1997). This means, that cytokines levels may vary considerably even between healthy individuals.

Engebretson et al (2002) concluded that GCF IL-1β expression is in part a host trait, and not strictly a function of clinical parameters. They speculated that carriage of the periodontitis associated genotype caused a "left shift" of the normal IL-1β expression pattern, meaning that increased IL-1 expression would be greater in each probing depth category. Thus, a postulated genetic mechanism might explain this increased expression of the important inflammatory mediator, IL-1β. It is likely that the nature of the antigen-presenting cell is fundamental in determining the nature of the cytokine profile (Seymour and Gemmell, 2001).

Age also influences the levels of IL-1β. Tsalikis et al (2002) have demonstrated that IL-1β levels observed in the young adults group are lower than those observed in the older age group, which could be related to the less pronounced clinical signs observed during the development of gingivitis in the younger subjects.

TNF-α has also been detected in GCF of healthy periodontal subjects, with a reported amount on filter strip per site ranging from 0.2 to 998.8 fmol with a median value of 3.7 fmol (Rossomando et al, 1990). To the contrary, PGE_2 is low or non-detectable in the crevicular fluid of healthy individuals.

Diseased Periodontium

Several authors have demonstrated higher levels of cytokines in inflamed periodontal tissues compared to healthy sites. Charon et al (1982) were the first to demonstrate that GCF from inflamed sites of gingivitis patients had increased IL-1β activity levels. Subsequent studies, Hou et al (1995), Yavuzyilmaz et al (1995) and Ishihara et al (1997), showed increased levels of IL-1 in the GCF with increasing inflammation and probing depth. Also the GCF IL-1β level was shown to increase several folds (7 to 12) during experimental gingivitis in humans (Heasman et al, 1993). Several other studies also pointed toward higher IL-1β levels in patients with periodontitis compared to gingivitis (Figueredo et al, 1999; Faizuddin et al, 2003).

The values of IL-1β obtained in patients with gingivitis range between 20 pg/ml and 132 pg/ml (Faizuddin et al, 2003). This wide range may be attributed to the variation in bacterial plaque accumulation or variation in subsequent inflammation due to plaque. However, another possibility, put forth by Kinane et al (1992), is the inherent variation in the ability to produce IL-1β and that could result in heterogenous responses accounting to intraspecies differences.

When comparing the values obtained in patients with gingivitis to those with periodontitis, a significant overlap exists having nearly similar levels of IL-1β (ranging between 102 pg/ml and 130 pg/ml). This can probably be explained by the conclusions drawn by Shapira et al (1994), who in their study have stated that the inter individual differences in mediator secretion may result from intrinsic differences between different patient groups. Differences in different forms of periodontal disease might be the result of the activity of different combinations of inflammatory mediators, suggesting heterogeneity of periodontal diseases.

It is important to notice, that most studies did not find any correlation between clinical findings and levels of IL-1β (Wilton et al, 1992; Wilton et al, 1993). Likewise, Figueredo et al (1999) in their study failed to find any significant difference in concentrations of GCF IL-1β between deep and shallow pockets of the same patient. They suggested that the concentrations of IL-1β are more a characteristic of an individual patient and less a result of the inflammatory state of the sampled site.

To the contrary, Giannopoulou et al (2003) observed marked differences of IL-1β in different disease category groups, as compared to a healthy group; a 3-fold increase was noticed in the gingivitis patients, a 6-fold increase in the adult periodontitis patients, and an almost 9-fold increase in the early-onset periodontitis group. Increased levels of IL-1β were also found in GCF from active, as compared to inactive sites, suggesting that this proinflammatory cytokine may serve as possible indicator of disease activity in refractory periodontitis (Lee et al, 1995). These differences in total amounts of cytokines may be useful in distinguishing between different forms of periodontal diseases.

Rossomando et al (1990) detected an increase in TNF-α levels in GCF from periodontal pockets. In addition, transcripts of its gene and its protein were detected at the cellular level in inflamed gingival tissues (Matsuki et al,1992). It was suggested, that TNF-α may be a marker of early inflammatory activity, because of its patternless distribution within the individuals and the lack of correlation with clinical parameters (Rossomando et al, 1990). TNF-α cannot always be retrieved from GCF. It was shown to be present in about 21-50% of examined sites (Rossomando et al, 1990, Yavuzyilmaz et al, 1995).

Yavuzyilmaz et al (1995) studied the GCF profile of TNF-α in patients with severe and rapidly progressive periodontitis and found mean TNF-α levels of 3.20±1.39 pg/ml. Bostrom et al (1998) examined the effect of smoking on GCF TNF-α levels. They showed that the TNF-α content was significantly increased in current smokers as compared to non-smokers (moderate to severe periodontal disease), with median GCF levels of 61.0 pg/ml for current smokers, 51.0 pg/ml for former smokers, and 12.0 pg/ml for non-smokers.

PGE_2 is easily detected in the GCF and increases 2- to 3-fold in gingivitis and periodontitis, relative to health. It increases another 5- to 6-fold during periods of active disease progression, as determined by longitudinal attachment loss (Offenbacher et al, 1993a; Offenbacher et al, 1993b). GCF PGE_2 levels increase prior to attachment level changes and can be used as a screening test to predict future attachment loss (Offenbacher et al, 1986). Although mean GCF PGE_2 level for a patient can be used to assess the patient risk for future attachment loss, with an overall predictive value of 0.92 to 0.95, the use of site's PGE_2 value as a site-specific indicator for adult periodontitis, is more complex (Offenbacher et al, 1993a; Offenbacher et al, 1993b). It was suggested that a PGE_2 level of 300 ng/ml GCF reflects an underlying disease activity of 0.5 to 1.0 mm of attachment loss/year (Offenbacher et al, 1993b).

PGE_2 is detected in higher levels in inflamed gingival tissue and GCF proportional to the severity of periodontal disease (Goodson et al, 1974; Offenbacher et al, 1983; Offenbacher et al, 1989; Ohm et al, 1984). Offenbacher et al (1989), using experimental periodontitis model in animals, reported that GCF PGE_2 and $PGF_{2\alpha}$ levels tended to increase at 3 months, reached a maximum level at 6 months, and returned to baseline values at 12. This was confirmed in a model of experimental gingivitis, where GCF PGE_2 levels increased at 4 weeks following the cessation of oral hygiene procedures (Heasman et al, 1993). Other studies have also confirmed that GCF PGE_2 levels are elevated in periodontitis compared to gingivitis (Gemmell et al, 1997; Tsai et al, 1998; Preshaw et al, 1999). Recent data suggests that the GCF PGE_2 levels are substantially higher in certain high risk patients such as refractory, early-onset periodontitis or diabetic patients (Offenbacher et al, 1994). It appears that the increased local GCF PGE_2 response observed in these patients is coincident with an up-regulated monocytic phenotype (MØ+) (Garrison et al, 1988; Shapira et al, 1994; Offenbacher et al, 1994; Salvi et al, 1997). Thus, even low levels of endotoxin challenge within the periodontal pocket seem to induce high levels of PGE_2 secretion at these patients. This suggests that the GCF PGE_2 level reflects the collective response of the patient's periodontium, not as a collection of sites which function independently, but rather as an organ which

reflects the patient's systemic response to local infection.

Preshaw and Heasman (2002) suggested that whatever the patient's phenotype (i.e., disease resistant or disease susceptible), there is a natural tendency for PGE_2 levels to gradually increase over time. In their modified model of disease pathogenesis, PGE_2 concentrations rise gradually in the periodontal tissues. A threshold (T_1) may then be reached at which attachment loss and/or bone loss occur, and clinical signs of disease progression become evident. This would correspond with the pooled GCF PGE_2 threshold value of 66.2 ng/ml, proposed, above which patients were significantly more likely to experience attachment loss. After this first threshold is reached, PGE_2 levels may continue to rise further, until a second threshold (T_2) is attained, at which time a negative feedback suppression of PGE_2 production occurs. This may have the effect of rapidly decreasing PGE_2 levels to 'baseline' values, thereby stabilizing attachment level and bone height. Individuals probably vary in their values of T_1 and T_2, which may, in part, explain some of the variations between patients regarding periodontal disease susceptibility. For example, in some individuals T_1 may never be reached, as negative feedback mechanisms may occur early (i.e., $T_1 > T_2$), and thus prevent PGE_2 concentrations from increasing sufficiently to cause attachment loss and bone loss.

GCF CYTOKINES AND PERIODONTAL THERAPY

Mechanical treatment of periodontitis, i.e. scaling and root planing, has been shown to lower GCF IL-1β levels (Masada et al, 1990; Reinhardt et al, 1993; Matsuki et al, 1993; Hou et al, 1995; Alexander et al, 1996; Engebretson et al, 2002). Reinhardt et al (1993) demonstrated decreased levels of IL-1α and IL-1β in shallow pockets and moderate pockets that had been treated non-surgically. Surprisingly, they also reported that sites surgically treated by papillary flap debridement continued to show elevated levels of both IL-1α and IL-1β after 6 months.

Engebretson et al (2002) showed that 2 weeks following scaling and root planning the levels of IL-1β in GCF were reduced in all patients. This reduction was more pronounced for patients with more severe periodontal disease. At 24 weeks, IL-1β continued to decrease for patients with less severe disease, while cytokine activity in individuals with more severe disease had rebounded and approached baseline levels. Hou et al (1995) also have shown that phase I periodontal therapy reduces the IL-1β in GCF, and claimed that this relationship may be useful in monitoring periodontal disease activity. Using tetracycline fibre therapy has also been shown to reduce IL-1β in GCF (Lamster et al, 1996).

Reports concerning GCF TNF-α levels following periodontal therapy are limited. Most studies show minimal changes of this cytokine. Engebretson et al (1999) showed that total TNF-α levels were doubled 3 weeks after treatment in patients positive for the periodontitis-associated genotype, while total GCF TNF-α level showed no significant changes in patients negative for the periodontitis-associated genotype.

Non-steroid anti-inflammatory drugs (NSAIDs) block PGE_2 synthase (COX). The suppression of PGE_2 synthesis with these drugs greatly diminishes attachment and bone loss, and thereby attenuates periodontal disease progression, both in animal and human models (Williams et al, 1989; Offenbacher et al, 1990, Offenbacher et al, 1992; Offenbacher et al, 1993a).

DISCUSSION

Periodontal diseases are chronic inflammatory diseases of the supporting structures of the teeth. They are triggered by periodontopathogens and the clinical outcome is highly influenced by the host local immune response (Kinane et al, 2001). Studies have suggested that the polarization of the local immune response, basically by T helper cells, may determine the stability or progression of the lesion (Mosmann and Coffman, 1989; Seymour and Gemmell, 2001). The polarized immune response may exhibit a Th1 pattern consisting of a predominantly pro-inflammatory cellular response, or a Th2 pattern, with anti-inflammatory characteristics and a predominantly humoral immune response. These distinct responses are mediated by characteristic cytokine patterns and involve the selective attraction of inflammatory cells to the site of response (Mosmann and Coffman, 1989; Jankovic et al, 2001).

IL-1 and TNF play a critical role in stimulating the innate host response and, in this capacity, prepare the host to defend itself against bacteria (Ferrante, 1992; Graves et al, 2000). A hypothesis was proposed that destructive periodontal disease may be due to dysregulation of these inhibitors, rather than an overproduction of IL-I and TNF-α per se (Howells, 1995). Regulation of the effects of these cytokines has been suggested for therapeutics used in tissue-destructive inflammatory diseases such as rheumatoid arthritis (Maini and Feldmann, 1996). This approach has also

been used for experimental periodontitis (Assuma et al, 1998; Graves et al, 1998; Delima et al, 2001).

Histologic studies, using soluble receptors as cytokine antagonists specific for IL-1 and TNF-α, have demonstrated an important role for IL-1 and TNF-α in destructive periodontitis (Graves et al, 1998; Delima et al, 2001). These findings showed that the cytokine antagonists, or blockers, inhibited: (a) the progression of the inflammatory front toward the osseous crest; (b) the recruitment of osteoclastic cells; (c) the loss of bone and connective tissue attachment in association with ligature-induced disease. Although associations have been established between levels of cytokines and presence of periodontal disease in general, large inter- and intra-individual variations suggest that these parameters are influenced by a multitude of other factors which, so far, have been poorly quantified. Several patient subgroups appear to have abnormally high monocytic responses with regard to the level of IL-1β, PGE_2, or TNF-α secreted, as compared to healthy controls or other periodontitis patients. In these high-risk patient subcategories the monocytic dose response curve has "shifted to the left" so that low dosages of LPS challenge result in 3- to 10-fold excess secretion of inflammatory mediators (a hyper-responsive monocytic trait) (Payne et al, 1993; Offenbacher et al, 1994; Shapira et al, 1994; Salvi et al, 1997).

It is clear from the aforementioned studies, that GCF level of these markers is not influenced only by the disease state of the periodontium. Variations of the IL-1 gene cluster have been proposed as genetic modifiers in a number of inflammatory diseases (Engebretson, 1999). Polymorphisms in the IL-1 gene cluster have been associated with an increased risk of developing certain diseases, including periodontal disease.

Armitage et al (2000) examined the prevalence of a periodontitis-associated IL-1 composite genotype in individuals of Chinese heritage. They concluded that the prevalences of both IL-1A and IL-1B polymorphisms are dramatically lower in Chinese than those reported for Europeans, which raises the question of the usefulness of the allele 2 composite genotype as a method for determining the susceptibility to adult periodontitis of Chinese patients or other populations. In another study, Engebretson et al (2002) concluded that GCF IL-1β expression is in part a host trait, and not strictly a function of clinical parameters. They speculated that carriage of the periodontitis associated genotype caused a "left shift" of the normal IL-1β expression pattern, meaning that increased IL-1β expression would be greater in each probing depth category. Thus, a postulated genetic mechanism might explain the increased expression of this important inflammatory mediator, IL-1β. Kinane et al (1999) analyzed the genetic polymorphisms at the interleukin-10 and tumour necrosis factor loci in early-onset periodontitis, but they could not demonstrate any link between the gene polymorphism and early-onset periodontitis.

Thus, genotyping must also be performed to rule out genetic bias.

CONCLUSIONS

GCF cytokine profiles vary with respect to when sampling was performed and the frequency of bone-resorptive active events. In this context, it is suggested that some markers might be associated with the early events of disease initiation, while other markers might be associated with chronic inflammation, during which events of tissue degradation occur. Monitoring of PGE_2, IL-1β and TNF-α levels in GCF may not only be a useful tool for monitoring disease activity, but may also help monitor the effectiveness of different treatment modalities. Therefore, standard levels of normal GCF markers should be established in the different populations.

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