Induction of Prostaglandin Release from Macrophages by Bacterial Endotoxin

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This review summarizes the role of the monocytic responses to lipopolysaccharide as it relates to periodontal disease severity. Data are presented which illustrate that the levels of prostaglandin E_2 (PGE_2) secreted by systemic peripheral blood monocytes in culture, in the presence of bacterial endotoxins, are highly correlated with the levels observed in the gingival crevicular fluid. Furthermore, the different periodontal diagnostic categories have varying levels of monocytic and crevicular fluid PGE_2, in juxtaposition with clinical disease severity. These data are consistent with the concept that there is close synchrony between the systemic responsiveness of peripheral blood monocytes with regard to prostanoid synthesis and the local levels of mediator present within the gingival crevice.

In recent years an altered central dogma regarding the pathogenesis of periodontitis has emerged [1, 2]. Periodontal diseases are widespread, but severe disease appears to affect only 7%–14% of the population [3]. Periodontitis is multifactorial in nature with regard to potential etiologic factors and effect modifiers. Since the classic 1965 article by Löe et al. [4], the microbial plaque has been considered the cause of periodontal diseases. Although certain gram-positive organisms have been associated with disease, the preponderance of data indicates that the gram-negative anaerobic species are the dominant pathogens [5]. However, recent data support the concept that microbial plaque is a necessary etiologic factor that is at the origin of the causality pathway but it is not sufficient to explain the presence, absence, or severity of disease. It is likely that one or more virulent clonal types of periodontal pathogens within the microbiota effectively evade host defenses to present an invasive challenge. Once the defenses are challenged, however, the quality of the host immunoinflammatory response appears to ultimately determine the severity and extent of disease expression.

The difference among people in terms of the nature of the immunoinflammatory response is a potential determinant of the variability in the expression of disease observed within the population. The current concepts of pathogenesis have extended the model from a plaque-dominated process to one in which the host response emerges as the dominant effect modifier in disease expression. Recent molecular and cellular data have improved our understanding of the inflammatory and immune mechanisms that result in the destruction of connective tissue, including ligament and bone. This sequence can be considered a central pathway that is common to most forms of disease but is activated or inhibited to varying degrees by various components of the host response and exposure factors. This pathway can be activated in cyclic fashion, as manifested by clinical progression or remission of disease activity. The central pathway that links microbial exposure to connective tissue destruction involves monocytic (Mϕ) activation.

In this model the monocytic activation by lipopolysaccharide (LPS; endotoxin) is a critical regulatory event that ultimately modulates connective tissue catabolism. The purpose of this article is to summarize the data regarding the role of monocytic secretion of prostaglandin E_2 (PGE_2) in various forms of periodontal diseases. New data are shown linking the local monocytic production of PGE_2 within the periodontal tissues (as measured in the gingival crevicular fluid, or GCF) and the responsiveness of systemic monocytes (as determined with use of peripheral blood monocytes in culture). Evidence is provided to suggest that the monocytic responsiveness to LPS, as reflected in the amount of PGE_2 secreted, may be a key regulator of disease expression. Furthermore, the magnitude of the monocytic responsiveness is simultaneously expressed locally and systemically. This coordination of monocytic conditions in the periodontal and systemic blood compartments suggests a systemic origin for the observed local effects.

LPS → Mϕ-Cytokine → Periodontal Disease

LPS binds in the crevicular fluid or serum to a high-affinity LPS-binding protein; this complex, in turn, binds to monocytic CD14, which activates monocytes. The CD14 receptor is a glycerolphosphatidyl inositol–anchored protein on the external surface of the monocyte cell membrane that serves as a high-

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affinity receptor for the LPS–LPS binding protein complex. When activated CD14 initiates a complex signaling cascade, phospholipase A_2 is activated to release arachidonic acid plus lysophospholipids from membrane phospholipid pools. Arachidonic acid is subsequently converted by the action of a cyclooxygenase (PGH_2 synthase) to PGE_2, which appears extracellularly. Transcriptional regulatory factors are activated and ultimately result in the transcription, synthesis, and secretion of several proinflammatory molecules, such as IL-1β, TNF-α, and IL-6. These four mediators target monocytes and fibroblasts to secrete matrix metalloproteinases to degrade collagen and glycosaminoglycans, thereby destroying the ligament and extracellular matrix. These mediators also act directly on bone cells to induce osteoclast formation and activation.

Clinically, activation of this pathway ultimately results in increased attachment and bone loss. Activation of this pathway can be detected biochemically, prior to any clinical sign of periodontal disease progression. Furthermore, experimental disruption in the central components of this host pathway abates clinical disease progression [4]. It should be emphasized that this description of an LPS-triggered cascade of events is a great oversimplification of a complex series of occurrences and divergent pathways.

If one is to test this model of pathogenesis, then logically the differences in disease expression observed in different people should be reflected in the relative degree of pathway activation. In this report we focus on the monocytic release of PGE_2 in response to LPS, examining the differences in PGE_2-release profiles among patients with various forms of disease and in health.

PGE_2 and Periodontal Disease Activity and Severity

Since original reports in 1986, considerable additional data have confirmed that the PGE_2 level within the gingival crevicular fluid can be considered a reliable biochemical marker for periodontal disease activity and severity [6–9]. During the induction of experimental gingivitis in humans, there is an increase in PGE_2 levels in GCF (GCF-PGE_2 levels), from 20.5 $\pm$ 7.6 ng/mL (mean $\pm$ SE, periodontal health) to 53.5 ng/mL after 4 weeks [10]. This GCF-PGE_2 level determined at 4 weeks is comparable to that measured cross-sectionally among patients with adult periodontitis (AP; 58.7 $\pm$ 11.2 ng/mL) [10, 11] and is coincident with the attainment of clinical inflammation. Thus, in a cross-sectional comparison, the GCF-PGE_2 level expressed as a mean patient value does not seem to be a sensitive discriminator between established gingivitis and stable or nonprogressing AP.

Furthermore, if one considers only shallow periodontal sites (i.e., 0–3 mm) in patients with AP, the GCF-PGE_2 levels are significantly higher than those of healthy subjects (49.0 $\pm$ 5.2 ng/mL vs. 20.5 $\pm$ 7.6 ng/mL). Thus, periodontally healthy sites in patients with AP have higher GCF-PGE_2 levels than clinically healthy sites in periodontally healthy subjects, consistent with an altered microbial burden and increased susceptibility to further breakdown. As expected, at moderate to deep (i.e., 4–11 mm) periodontal sites in patients with AP, there is a significant elevation in GCF-PGE_2 levels as compared with those at shallow sites (71.0 $\pm$ 11.8 ng/mL vs. 49.0 $\pm$ 5.2 ng/mL). Furthermore, the mean GCF-PGE_2 level in patients in whom periodontal disease progression is detected, as defined by attachment loss of $\geqslant$3 mm at one or more sites over a 6-month period after initial therapy, is significantly higher than in subjects who have no clinical signs of disease activity (113.4 $\pm$ 9.0 ng/mL vs. 50.1 $\pm$ 7.1 ng/mL; $P < .00001$).

This observation holds true at the time of detection of attachment loss as well as 6 months before this time point. Furthermore, at sites undergoing periodontal attachment loss, the GCF-PGE_2 level is 305.6 $\pm$ 56.5 ng/mL, showing a fivefold to sixfold elevation in comparison with the level at nonprogressing sites. Thus, biochemically the GCF-PGE_2 level represents a continuum from health to progressing disease as the PGE_2 level increases from ~30 ng/mL to 300 ng/mL, a 10-fold increase in mediator level. Levels above 60–100 ng/mL reflect increased risk or subclinical destruction. However, these values refer to normal, otherwise healthy people who have either gingivitis or AP. Some patients, such as those with diabetes or early onset disease, appear to have an altered GCF-PGE_2 profile, which is consistent with increased risk for disease progression and a parallel upregulated systemic monocytic state.

GCF-PGE_2 in High-Risk Patients

An exaggerated GCF-PGE_2 inflammatory response has been documented in several high-risk patient groups, as compared to that among otherwise healthy patients with gingivitis and AP [9, 11]. These high-risk categories include patients with early onset periodontitis (EOP), refractory periodontitis (RefP; American Academy of Periodontology [AAP] type V), insulin-dependent diabetes mellitus (IDDM)–associated AP (IDDM-AP), and terminal dentition periodontitis (TDP, i.e., extremely severe periodontitis requiring multiple extractions). Thus, these subjects with different forms of disease seemed to share a propensity for developing severe forms of periodontal disease and also had elevated GCF-PGE_2 levels. This observation prompted us to examine whether the production of PGE_2 by peripheral blood monocytes from these patients may be enhanced in response to LPS challenge, as compared with such production in periodontally healthy individuals.

The underlying hypothesis was that monocytes from patients who had more severe disease or were actively losing attachment at the time of sampling would secrete more inflammatory markers (with PGE_2 as a representative mediator) than would monocytes from subjects with less severe forms of disease, inactive disease, or no disease. We hypothesized that this would be reflected as a shift to the left in the dose-response curve when examining the PGE_2 response vs. the LPS dosage; thus, at any

level of LPS (including zero) patients with more severe disease would secrete more PGE_2 per monocyte.

We also hypothesized that the systemic responsiveness of these monocytes would regulate the amount of PGE_2 produced locally in the periodontal pocket, as measured in the GCF. The half-life of gingival monocytic cells is about 18 days. Thus, any systemic basal responsiveness would be expected to eventually be manifest locally, since LPS is an omnipresent challenge in the subgingival periodontal pocket. For this reason, we also examined the potential association between the levels present within the GCF compartment and that produced by monocytes in culture upon LPS stimulation at a fixed suboptimal concentration (0.03 μg/mL). In effect, we sought to test our 1993 hypothesis that the patient's monocytic mediator response to LPS challenge was a key determinant of disease expression [12].

Materials and Methods

Patient Selection

This report includes data from a total of 209 subjects divided into 7 main diagnostic categories: health ($n = 34$), AP ($n = 66$), EOP ($n = 22$), RefP ($n = 24$), IDDM-AP ($n = 34$), TDP ($n = 16$; discussed below), and paroxysmal nocturnal hemoglobinuria ($n = 13$). All patients except those with IDDM-AP or paroxysmal nocturnal hemoglobinuria were free of systemic conditions or medications known to affect inflammatory or periodontal status. Some data from most of these patients have been reported previously [6–8, 10–13]. This report provides a reanalysis of previous data and a master compilation of existing patients, including new patients added since our original publications. Specifically, data demonstrating dose-response curves between LPS concentration and monocytic secretion of PGE_2 have been published for 21 healthy patients [8], 21 patients with AP [8], 25 patients with IDDM-AP [8], and 16 patients with TDP [9]. For the purposes of this report, data concerning monocytic release of PGE_2 are shown for the different patient groups using a single, submaximal LPS concentration for stimulating Mϕ in culture, adding new patients to the previously published data set.

The subjects with EOP include 22 patients who met the criteria described by Gunsolley et al. [14]. Patients with AP were subdivided according to type of disease (AAP types I to IV) and by the pooling of types I and II ($n = 36$) as well as types III and IV ($n = 30$), on the basis of similarities in GCF and monocytic secretory patterns. RefP (AAP type V) is defined by Collins et al. [13]. Patients with IDDM-AP (AAP types I and II [$n = 9$] and types III and IV [$n = 25$]) are characterized in detail elsewhere [14, 15]. Recently, a new periodontitis category has been suggested: TDP, as adapted from the prosthodontic literature [9]. This refers to extremely severe forms of AP and EOP that are at a transition stage between severe periodontitis and edentulism. Basically, these patients present with multiple teeth that are hopelessly involved with periodontitis and require extractions. The subjects with TDP included eight with AP and eight with generalized EOP, according to the EOP criteria of Gunsolley et al. [14]. Finally, the last group of interest included 13 patients with paroxysmal nocturnal hemoglobinuria [15], a condition in which patients lack monocytic CD14 because of an impaired ability to synthesize the glycerolphosphatidyl inositol anchor protein that attaches the CD14 receptor onto the cell membrane. Thus, monocytes from these individuals lack the membrane-bound, high-affinity LPS–LPS binding protein receptor, CD14. As will be shown and appears elsewhere [15], monocytes from these individuals have an impaired ability to respond to bacterial endotoxin with respect to mediator production.

GCF Sampling and Analysis

GCF was collected with Periopaper filter strips (Pro Flow, Amityville, NY), and the volume was quantitated with a calibrated Periotron 6000 instrument (Pro Flow). The concentration of PGE_2 within the GCF was determined by means of RIAs or ELISAs. Since the procedures for GCF collection, storage, and analysis have previously been described [8, 16–18], only a brief summary of the sampling technique and analysis is presented. The interproximal area between the two most posterior teeth in each quadrant was selected for GCF sampling. Four filter strips, one in each quadrant, were used for each patient. The preselected sites were isolated with cotton rolls and air-dried, and then the GCF filter strips were placed into the periodontal pocket until visibly moistened. After their removal, the volume was determined with the Periotron 6000. Immediately afterward, the collected samples were wrapped in aluminum foil, placed into screw-top cryovials, and transferred into liquid nitrogen. All GCF samples were maintained in liquid nitrogen until the assays were performed. Prior to assay, each filter strip was eluted and independently analyzed for PGE_2, expressed as a GCF-PGE_2 concentration in ng/mL. The mean GCF-PGE_2 concentration for each patient represents the average for four sites.

Monocyte Isolation and Stimulation with LPS

The monocytic secretion of PGE_2 in the peripheral blood of each patient was evaluated following monocyte isolation, culturing in the presence of LPS, and determination of PGE_2 concentration in the supernatant, with use of the methods described earlier [8, 9, 16]. A standard venipuncture technique was used to collect ~40 mL of whole blood in heparinized tubes, and mononuclear cells were isolated with use of Ficoll-Hypaque density gradients (Sigma Chemical, St. Louis), followed by adherence to plastic culture wells. Mononuclear cells

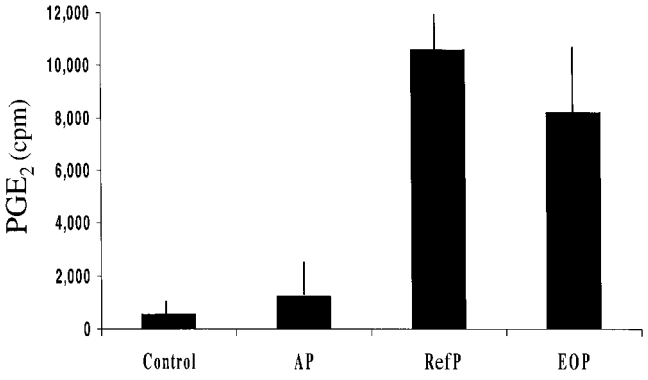

Figure 1. Basal (no lipopolysaccharide challenge) secretion of prostaglandin E_2 (PGE_2, measured in counts per minute [cpm]) from isolated peripheral blood monocytes from periodontally healthy controls (*Control; n* = 11) and from patients with adult periodontitis (*AP; n* = 5), refractory periodontitis (*RefP; n* = 8), and early onset periodontitis (*EOP; n* = 10). Unstimulated monocytes from subjects with RefP and EOP secrete significantly higher amounts of PGE_2 than do those from pooled control subjects and patients with AP (P = .01 for RefP vs. AP + control and P = .05 for EOP vs. AP + control). Standard error bars are shown.

were resuspended in RPMI medium in the presence of a 10% pooled human antibody serum source of LPS-binding protein and 1% penicillin/streptomycin at 37°C in 5% CO_2. Isolated monocytes were placed in short-term culture with varying amounts of LPS (*Porphyromonas gingivalis* and *Escherichia coli,* over a dose-response range of 0.003 to 3.0 μg/mL) for PGE_2 quantitation. The supernatants were harvested after overnight incubation (12–18 hours) and analyzed by RIA or ELISA for the amount of PGE_2 present.

In certain experiments, monocytes were preincubated with tritiated arachidonate–human serum albumin conjugate. This resulted in uptake of arachidonate into preexistent phospholipid pools. Following LPS stimulation, supernatants from these labeled cells were subjected to reverse-phase high-pressure liquid chromatography (HPLC), and the amount of radiolabeled PGE_2 was determined with an in-line scintillation counter, as described earlier [19]. In brief, adherent monocytes were released (0.01 m*M* EDTA-PBS [pH, 7.4] at 4°C) and pelleted. Cells were prelabeled by incubating with ^{3}H arachidonic acid–human serum albumin conjugate in RPMI medium, washed, and replated at 10^8 cells/mL in RPMI medium supplemented with 10% pooled antibody serum (Sigma, St. Louis) and 1% penicillin/streptomycin. After incubation, monocyte supernatants were extracted as described by Powell [19]. To each sample, 10,000 counts per minute (cpm) of ^{3}H-6K-PGF1α was added as internal standard. To 0.5 mL of media, 1.0 mL of ethanol was added and centrifuged.

The pellet was washed with 0.5 mL of ethanol, the supernatants were pooled, and the sample was adjusted with water to 15% ethanol, with a final volume of 15 mL. Acetic acid was added to a pH of 3.0, and the sample was passed over a preconditioned C_{18} column (Sep-pak, Waters Inc., Marlborough, MA). The column was sequentially washed with 20.0 mL of 15% ethanol, 20 mL of petroleum ether, and the prostanoids eluted with 10 mL of methyl formate. The methyl formate was evaporated to dryness under nitrogen and dissolved in HPLC buffer (30% acetonitrite and 17-m*M* H_3PO_4). The PGE_2 was resolved isocratically on a reverse-phase (RP) Speri-5, RP-18, 100 × 4.6-mm, 5-μ column after passing through an RP-18, 7 precolumn at a flow rate of 1 mL/min. Prostaglandins were detected at 192 nm and quantitated by an in-line scintillation counter (Flow-one, Inus Systems, Tampa, FL). Peak areas were integrated, corrected for recovery, and expressed relative to cell number or DNA content for comparisons.

Results

Monocyte Secretion of PGE_2 in Unstimulated and LPS-Stimulated Cultures

In a subset of patients, monocytes were preincubated in the presence of tritiated arachidonic acid, and the release of PGE_2 was monitored by extraction, separation by HPLC, and quantitation by scintillation counting. The release of radioactive PGE_2 into the media from unstimulated monocytes is shown in figure 1. As can be seen, the basal secretion of PGE_2 in patients with EOP and RefP is significantly higher than that in healthy subjects or patients with AP. There is no difference in the level of PGE_2 secreted in healthy controls vs. patients with AP. Thus, freshly isolated, unstimulated monocytes in subjects with EOP or RefP appear to be ''activated or primed'' in the peripheral blood of these subjects, as compared with such monocytes in healthy subjects or those with AP. This is reflected in the approximate ninefold to 10-fold elevation of monocytic PGE_2 in the absence of exogenous LPS.

Furthermore, monocytes from patients with EOP or RefP secrete more PGE_2 in response to LPS challenge throughout the entire dose-response curve (figure 2), as compared to AP or healthy controls. In these data, the elevated PGE_2 secretion throughout the LPS dose-response appears to be largely due to differences in basal secretion. In this early experiment and in subsequent experiments, no significant difference in the monocytic release of PGE_2 has been observed in terms of the response in healthy patients or those with gingivitis vs. patients with moderate to severe AP. However, in both EOP and RefP, there is an increase in the maximum level secreted (about twofold to threefold) and a shift to the left in the dose-response curve. This indicates not only that monocytes from these two patient groups secrete higher levels of PGE_2 but that low levels of LPS, which are below the activation threshold of control or AP monocytes, trigger PGE_2 secretion in these subjects. Thus, even relatively low levels of endotoxin might be expected to result in PGE_2 secretion in the GCF in these individuals.

This early observation led to a series of experiments involving many different groups of patients to determine whether this

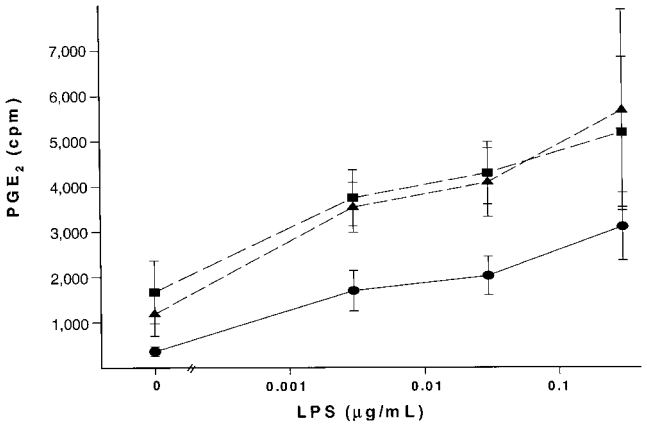

Figure 2. Systemic monocytic prostaglandin E_2 (PGE_2) secretion from patients with gingivitis and adult periodontitis (American Academy of Periodontology [AAP] stages I–IV; $n = 16$; ●), refractory periodontitis (AAP stage V; $n = 8$; ■), and early onset periodontitis ($n = 10$; ▲). There is a significant difference over the entire dose-response curve (with 0–3.0-μg/mL doses of LPS [lipopolysaccharide isolated from *Porphyromonas gingivalis*]) when monocytic PGE_2 secretions from patients with gingivitis and adult periodontitis are compared with those from patients with refractory and early onset periodontitis. Standard error bars are shown. cmp = counts per minute.

systemic monocyte responsiveness trait, as determined by the amount of PGE_2 secreted in culture, could be correlated to the severity of the condition and the level of PGE_2 present within the GCF. This experiment was conducted by generating LPS-dose vs. PGE_2-response curves for each subject and simultaneously measuring the PGE_2 level within the GCF, averaging over four sites per subject. To permit easy comparison among patients, the data are shown for the monocytic PGE_2 level at only one suboptimal LPS concentration (0.03 μg/mL) vs. the GCF-PGE_2 level. These data are developed sequentially in figure 3. It should be noted that the monocytic secretion of IL-1β and TNF-α has also been investigated in some of these patients and is reported on elsewhere [8, 9, 16]. These data are not presented in this report but in general show the same trend as PGE_2 responses.

Monocytic and GCF-PGE_2 Levels in Otherwise Healthy Adults with Periodontitis and in CD14-Deficient Patients

Figure 3 shows the amount of monocytic PGE_2 secreted in response to *P. gingivalis* LPS (0.03 μg/mL) in healthy persons and in patients with AP (AAP stages I–II or III–IV) or paroxysmal nocturnal hemoglobinuria. As can be seen, the amount of monocytic PGE_2 secreted in subjects with AP at AAP stages I–II (61.0 ± 4.6 ng/mL) or III–IV (68.4 ± 7.4 ng/mL) and in healthy individuals (65.9 ± 9.0 ng/mL) is virtually identical. We have previously referred to this response as a normal monocytic secretory trait, or $M\phi^N$. In comparison, the patients with paroxysmal nocturnal hemoglobinuria have a significantly reduced monocytic secretory capacity and secrete only 28.5 ± 5.8 ng of PGE_2 per mL ($P < .00002$, in comparison with the level for pooled $M\phi^N$ subjects). This is designated as a $M\phi^-$ trait for these CD14-deficient subjects. The GCF-PGE_2 levels for the $M\phi^N$ subjects are consistent with the local increase in bacterial burden associated with AP of AAP types I–II (44.4 ± 5.8 ng/mL) and III–IV (58.7 ± 11.2 ng/mL).

This finding suggests that the significantly lower GCF-PGE_2 levels seen in healthy persons (22.5 ± 4.8 ng/mL) than in pooled subjects with AP of AAP stages I–IV ($P < .002$) are a reflection of the lower LPS burden associated with optimal periodontal health. It is interesting that a reasonable linear correlation exists among the patients and that the healthy individuals are outliers at this particular LPS concentration. This suggests a significant positive association between the GCF-PGE_2 level and the systemic monocytic response. This concept is further substantiated by additional patient data.

Monocytic and GCF-PGE_2 Levels in Patients with RefP and EOP

In figure 3 two additional patient groups, those with RefP and EOP, are considered. These more aggressive forms of

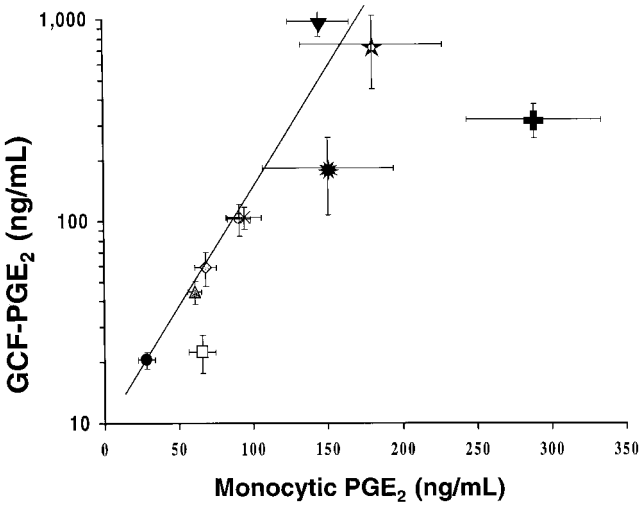

Figure 3. Relationship between systemic monocytic prostaglandin E_2 (PGE_2) release upon challenge with lipopolysaccharide (0.03 μg/mL, from *Porphyromonas gingivalis*) and local levels of PGE_2 in gingival crevicular fluid (GCF) in CD14-deficient subjects (●; paroxysmal nocturnal hemoglobinuria; $n = 13$); in periodontally healthy patients (▨; $n = 34$); in patients with gingivitis/mild periodontitis (△; American Academy of Periodontology [AAP] stage I or II; $n = 36$), moderate/severe adult periodontitis (◊; AAP stage III or IV; $n = 30$), refractory periodontitis (○; AAP stage V; $n = 24$), or early onset periodontitis (×; EOP; $n = 22$); in patients with insulin-dependent diabetes mellitus and either gingivitis/mild AP (✷; AAP stage I or II; $n = 10$) or moderate/severe AP (✚; AAP stage III or IV; $n = 29$); and in patients with terminal-dentition-stage AP (▼; $n = 8$) or terminal-dentition-stage EOP (☆; $n = 8$). Standard error bars are shown.

periodontal disease are associated with a shift in the monocytic responses. The amount of PGE_2 secreted by monocytes from subjects with RefP (94.4 ± 11.7 ng/mL) or EOP (90.0 ± 7.6 ng/mL) is significantly greater than that in healthy subjects or patients with AP ($P < .01$, vs. pooled $M\phi^N$ subjects). This systemic monocytic trait appears stable, since the level of secretion remains elevated in controls with AP following treatment [6], and the term hyperresponsive monocytic trait (or $M\phi^+$ trait) has been used [11] to characterize this upregulated systemic monocyte characteristic.

The highly significant elevations in levels of GCF-PGE_2 (RefP: 104.0 ± 13.1 ng/mL; EOP: 102.6 ± 18.4 ng/mL) relative to those in pooled subjects with AP of AAP stages I–IV ($P < .00006$ for RefP and $P < .0007$ for EOP) also are consistent with the rapidly progressive and active clinical courses of these conditions. These subjects are at high risk for clinical breakdown compared with the risk for subjects with AP. It is interesting that the ratio between systemic and local responses remains identical for these subjects, in contrast with the ratios for subjects with AP. This can be noted in figure 3, in which all of these diseased subjects fall on the same linear regression line. In this regard, the observed GCF-PGE_2 level might be anticipated on the basis of the systemic monocytic response trait.

The combination of these five patient groups establishes a clear, positive association between local and systemic PGE_2 responses, as evident in figure 3. Using the mean values from these five patient groups as point estimates to compute the linear regression curve for the association between GCF-PGE_2 and monocytic (''mono-'') PGE_2 (at 0.03 μg of *P. gingivalis* LPS per mL) provides the following equation:

$$\text{Log}_{10}[\text{GCF-PGE}_2\ \text{ng/mL}] = 0.01 \times [\text{mono-PGE}_2\ \text{ng/mL}] + 1.0, \text{ with } r = 0.997 \text{ and } r^2 = 0.995\ (P = .0001).$$

Thus, incremental changes in the systemic monocytic response are reflected as exponential changes in the local GCF-PGE_2 levels.

Monocytic and GCF-PGE_2 Responses in TDP and IDDM-AP

Figure 3 also illustrates the local and systemic responses of patients with severe AP and generalized EOP at a terminal dentition (TD) stage. The periodontal status of these subjects is reflected in the severity scores (mean clinical attachment levels, only among sites for which the scores were ⩾4 mm) of 6.03 ± 0.33 mm for TD-AP and 6.11 ± 0.42 mm for TD-EOP. Recent data by Salvi et al. [9] have provided evidence that patients with TD-AP and TD-EOP have exaggerated GCF and monocytic secretions of several inflammatory mediators, including PGE_2, as compared with secretions in patients with AAP type IV periodontitis. Data from that investigation illustrate that the GCF has unexpectedly high levels of PGE_2 in both TD-AP (970.5 ± 154.7 ng/mL) and TD-EOP (749.4 ± 297.4 ng/mL) groups, as compared with levels among subjects with moderate/severe AP (58.7 ± 11.2 ng/mL; $P < 10^{-5}$) or EOP (102.6 ± 18.4 ng/mL; $P < .001$). Furthermore, LPS-stimulated monocytic PGE_2 levels for both TD-AP (145.0 ± 20.7 ng/mL) and TD-EOP (180.6 ± 47.6 ng/mL) patients are greater than those for patients with moderate/severe AP (68.4 ± 7.4 ng/mL; $P < .001$) or EOP (90.0 ± 7.6 ng/mL; $P < .05$). It is interesting to note that the relationship between local and systemic PGE_2 response appears to maintain a similar ratio or slope as the severity of disease escalates.

Finally, figure 3 illustrates the responses of the subjects with IDDM-AP, subdivided by AAP stages (I–II and III–IV). These data from an earlier publication [8] demonstrate a significant 4.2-fold elevation in GCF-PGE_2 level in a comparison of diabetics with AP of AAP stage I–II (183.2 ± 76. ng/mL) to periodontally matched, nondiabetic subjects (43.3 ± 8.1 ng/mL) ($P = .003$). A higher, 7.4-fold elevation is observed in a comparison of GCF-PGE_2 levels for patients with IDDM-AP of AAP stage III–IV (319.1 ± 62.2 ng/mL) and those for periodontally matched nondiabetics (42.9 ± 11.9 ng/mL) ($P = .0002$). Among diabetics, the GCF-PGE_2 level is significantly elevated for those with moderate-severe periodontitis, as compared with that for patients with gingivitis/mild periodontitis ($P = .01$). Diabetics as a group show a 13.8-fold elevation in GCF-PGE_2 level over the level for periodontally healthy persons and a 6.6-fold elevation over that for nondiabetics with moderate-severe periodontitis ($P < .00001$). These data clearly suggest that IDDM results in a dramatic elevation in the GCF-PGE_2 levels, irrespective of periodontal status.

Discussion

Data regarding cultured peripheral blood monocytes from high-risk patients (with EOP, RefP, IDDM-AP, and TDP) suggest that the locally elevated GCF-PGE_2 levels are associated with a systemically upregulated monocytic PGE_2 trait. These observations confirm and extend previous investigations suggesting that patients with RefP [20, 21] and EOP [22] have considerably elevated monocytic PGE_2 levels as compared with those in patients with AP. In 1989 Garrison and Nichols [20] demonstrated that LPS-stimulated monocytes from ''periodontitis-susceptible'' subjects secreted twofold to threefold more PGE_2 than those from ''periodontitis-resistant'' patients. In this context the term *susceptible* was used to describe cases of RefP, whereas the term *resistant* referred to patients with untreated periodontitis who had poor plaque control and little or no disease. Shapira et al. [22] observed significantly greater basal levels (no LPS challenge) of monocytic inflammatory mediators, including PGE_2, in 28 patients with EOP than in periodontally healthy controls. Furthermore, these investigators found a consistent threefold to fivefold greater monocytic PGE_2 secretion throughout the LPS dose-response curve than that in control subjects. Thus, these earlier observations have now been replicated and expanded by the current report of 209 subjects,

and collectively they provide compelling evidence that the systemic, peripheral blood monocyte is upregulated with regard to its secretory capacity in conditions such as EOP, RefP, IDDM-AP, and TDP.

The hypersecretory phenotype ($M\phi^+$) occurs spontaneously and throughout the LPS dose-response range. The systemic monocyte in RefP and EOP appears to be ''primed'' in that it synthesizes greater amounts of PGE_2 in culture, even in the absence of LPS challenge, as compared with amounts synthesized in healthy controls or subjects with AP. Although not shown in figure 2, additional findings by Salvi et al. [8] indicate that systemic monocytes in subjects with IDDM also appear to be primed in the circulation. Freshly isolated unstimulated peripheral blood monocytes from patients with IDDM secrete higher amounts of PGE_2 than do those from nondiabetic controls. Basal monocytic PGE_2 levels in diabetics with gingivitis/mild periodontitis (75.0 $\pm$ 32.0 ng/mL) and moderate/severe periodontitis (104.0 $\pm$ 21.0 ng/mL) are higher than those in control subjects with moderate/severe AP (57.1 $\pm$ 5.6 ng/mL). Thus, patients with EOP, RefP, and IDDM-AP all appear to have basal or resting-state monocytic responses that are elevated with regard to PGE_2 production.

It is significant that AP, the most common periodontal condition (accounting for ~85% of all periodontal cases), is associated with a normal monocytic response ($M\phi^N$), as determined with or without LPS challenge. Thus, cross-sectionally it would appear that subjects with AP have no evidence of monocytic abnormalities or ''priming'' as a consequence of genetic background or environmental exposures such as infection, including periodontal disease. This would suggest that in AP, the microbial challenge that occurs systemically, albeit transient in nature, is not a sufficient challenge to cause an upregulated, steady-state priming of the systemic peripheral blood monocyte.

It is not clear from these cross-sectional data whether, during episodes of acute disease progression, patients with AP have a transiently upregulated monocyte phenotype. However, the close coupling of the local and systemic PGE_2 responses, as demonstrated in figure 3, would suggest that previously reported transient elevations of mean GCF-PGE_2 levels (to 113.4 $\pm$ 9.0 ng/mL) [6] that occur during longitudinal episodes of disease progression are associated with a monocytic response of 105 ng/mL (as computed with use of the regression function shown above). As can be seen, this value would theoretically place the monocytic response in cases of AP breakdown equal to that in cases of RefP and EOP involving $M\phi^+$ patients. On the basis of the elevated GCF-PGE_2 levels during disease progression, these data suggest that during these short periods of active attachment loss, the patients with AP probably have a transient $M\phi^+$ phenotype. This concept is further substantiated by the observed hyperresponsive shift in the monocytic trait in TDP cases, as discussed below.

Monocytes from the subjects with paroxysmal nocturnal hemoglobinuria who lack glycerolphosphatidyl inositol–anchored CD14 demonstrate a reduced PGE_2 secretory capacity, which is consistent with the established role of CD14 as a high-affinity LPS–LPS binding protein receptor. This $M\phi^-$ phenotype is logically a consequence of this membrane-receptor deficiency. Although a few of these subjects were taking systemic steroids to manage the clinical course of paroxysmal nocturnal hemoglobinuria, excluding these subjects from this dataset does not significantly alter the data, and the monocytic responses in this culture assay are not different in a comparison of patients taking steroids and those who were not (details appear in [15]).

In contrast to the molecular mechanisms responsible for the observed $M\phi^+$ trait in EOP, RefP and IDDM-AP are not presently understood. Nonetheless, several potential explanations have emerged and include the following.

(1) Mølvig and colleagues [23] have demonstrated stable interindividual differences in monocytic release of IL-1β, TNF-α, and PGE_2 in response to LPS. Thus, there is a wide spectrum in the magnitude of the inflammatory-mediator release among different individuals. This response trait appears to be relatively stable over short periods of time.

(2) One possible explanation for the large variance in the magnitude of mediator secretion in the normal population is that certain genetic polymorphisms are associated with a hypersecretory or hyposecretory monocytic trait (see [24, 25] for review). Several of these stable genetic polymorphisms have been associated with increased severity of infectious or inflammatory conditions, including cerebral malaria [25], mucocutaneous leishmaniasis [26], and psoriasis [27]. These polymorphisms have been identified for IL-1 [28] and TNF-α [29], both of which are capable of autocrine upregulation of monocytic PGE_2 secretion. Thus, elevated PGE_2 secretion may be an indirect indicator or consequence of enhanced release of IL-1 or TNF-α.

(3) Allelic variants in the IL-1 gene cluster result in altered IL-1β response, and certain polymorphisms may be related to certain severe forms of AP [30]. Certain environmental exposures, such as smoking, may override any genetic contribution to disease expression. It is not known whether the increased disease susceptibility and high PGE_2 levels in the four high-risk patient groups in this study are a consequence of a hypersecretory IL-1 and/or TNF-α polymorphism.

(4) Environmental exposures such as smoking have been demonstrated to enhance monocytic PGE_2 secretion [31]. Unfortunately, this important risk factor (see [32] for review) was not uniformly determined for each subject in this study. Many of the subjects with RefP and three of the 16 subjects with TD-AP were smokers. Thus, smoking is one important potential mechanism for induction of a steady-state $M\phi^+$ phenotype.

(5) Metabolic lipid dysregulation upregulates monocytic responses to LPS challenge [33, 34]. Specifically, high levels of low density lipoprotein prime monocytes to secrete elevated amounts of PGE_2 subsequent to challenge. Unfortunately, blood lipid chemistries were not included in our data collection.

(6) Advanced glycation end products (AGEs), which occur as a consequence of chronic hyperglycemia, have been demonstrated to target specific receptors on monocytes [35]. AGE-stimulated monocytes secrete higher levels of IL-1 and TNF-α in culture [36]. This is one possible mechanism for the observed $M\phi^+$ response in patients with IDDM-AP.

(7) Psychosocial stress has been shown to be a risk factor for disease progression [37, 38]. As a consequence of acute stress, elevated levels of vasoconstrictor peptides such as angiotensin II and endothelin-1 stimulate PGE_2 synthesis [39]. Other stressors, including oxidative stress and infection (via IFN-γ), also enhance the magnitude of the monocytic PGE_2 response [40].

From this brief summary of genetic and environmental exposures that may all have the potential to modulate the monocytic and local PGE_2 response, it appears that any number of possible modifiers may contribute to the observed findings. It is important to note that although these data include patients at different stages of disease progression, this is a cross-sectional survey and as such can only suggest an association between systemic responsiveness and local mediator levels and cannot establish a temporal cause-and-effect relationship. Nonetheless, it is remarkable that there is such a high degree of correlation of local and systemic responses. Because of the turnover of the monocytic cells within the gingiva, it seems reasonable to suggest that the responsiveness characteristic seen systemically ''drives'' or determines the local response with remarkable fidelity. However, TDP presumably is an end-stage clinical manifestation of AP and EOP. Subjects with TDP had unexpectedly high levels of inflammatory mediators. It is possible that this is a transient shift in responses. However, the chronicity of the condition may lead to a systemic upregulation as a direct consequence of the periodontal infection; that is, during active disease progression, the systemic ''dumping'' of LPS and cytokines such as TNF-α [41] may account for the extraordinarily high monocytic response trait.

It does not seem unreasonable to suggest that this severe periodontal infection represents a source of systemic sepsis and therefore that the local infection serves to drive the systemic response. Thus, in the TD state, this may be interpreted as an oral infection inducing a mild systemic sepsis that is subclinical in nature with regard to symptomatology but detectable by examination of monocytic priming.

In conclusion, these data provide evidence that high-risk periodontal patients (i.e., with EOP, RefP, IDDM-AP, and TDP) have a monocytic hypersecretory trait ($M\phi^+$) that predisposes to an exaggerated local and systemic inflammatory reaction in response to LPS challenge. Thus, the enhanced PGE_2 secretory capacity of peripheral blood monocytes offers a possible explanation for the increased likelihood of more severe periodontal disease in these patient groups. Further research should enable us to elucidate the underlying genetic and environmental mechanisms that contribute to the observed upregulated monocytic phenotype.

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