Differential plateletactivating factor synthesis by monocytes and polymorphonuclear leukocytes from subjects with localized aggressive periodontitis

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Background and Objective: Platelet-activating factor is elevated in localized aggressive periodontitis. We previously demonstrated that the elevated level of platelet-activating factor in localized aggressive periodontitis is at least partially attributable to low levels of platelet-activating factor acetylhydrolase, the enzyme that catabolizes platelet-activating factor. The objective of this study was to determine if platelet-activating factor synthesis was also elevated in localized aggressive periodontitis. To test this, platelet-activating factor synthesis was quantified in the monocytes and polymorphonuclear neutrophils of periodontally healthy patients and of subjects with localized aggressive periodontitis.

Material and Methods: Cells were labeled with [³H]acetate and treated with vehicle or stimulated with calcium ionophore A23187. Platelet-activating factor was extracted and quantified by scintillation counting.

Results: For both subject groups, resting monocytes and polymorphonuclear neutrophils produced platelet-activating factor, and calcium ionophore A23187 stimulated platelet-activating factor production in both cell types. However, calcium ionophore A23187-activated monocytes from subjects with localized aggressive periodontitis produced less platelet-activating factor than did activated periodontally healthy monocytes (p < 0.0001), suggesting an aberrant calcium ionophore A23187 response in monocytes from subjects with localized aggressive periodontitis. Indeed, when the data were expressed as fold induction of platelet-activating factor synthesis in response to calcium ionophore A23187, monocytes from subjects with localized aggressive periodontitis exhibited only a fourfold increase in platelet-activating factor synthesis, whereas calcium ionophore A23187-stimulated monocytes from periodontally healthy, chronic periodontitis and generalized aggressive periodontitis subjects produced ≈ 12 times more platelet-activating factor than did resting monocytes. In contrast, both resting and

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H. A. Schenkein², S. E. Barbour^{1,2} ¹Department of Biochemistry and ²Clinical Research Center for Periodontal Diseases, Virginia Commonwealth University, Richmond, VA, USA activated localized aggressive periodontitis polymorphonuclear neutrophils synthesized more platelet-activating factor than did periodontally healthy polymorphonuclear neutrophils.

Conclusion: These data suggest that high levels of platelet-activating factor in subjects with localized aggressive periodontitis result from both increased synthesis and reduced catabolism. While localized aggressive periodontitis polymorphonuclear neutrophils contribute to increased platelet-activating factor mass through synthesis, the contribution of monocytes is probably the result of reduced catabolism by platelet-activating factor acetylhydrolase.

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Localized aggressive periodontitis is an early onset form of periodontitis, characterized by aggressive destruction of the gingival tissues, the supporting periodontal ligaments and the alveolar bone (1,2). Localized aggressive periodontitis has a bacterial etiology, with infection of the gingival tissues by several bacterial pathogens, most commonly Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis (3,4). However, in addition to bacterial etiology, host immune and inflammatory factors are implicated in the pathogenesis and progression of localized aggressive periodontitis. For example, increased protective immune responses have also been observed in subjects with localized aggressive periodontitis, as demonstrated by the enhanced immunoglobulin G2 responses to oral pathogens that correlate with reduced severity of disease (5-7). In contrast, the polymorphonuclear neutrophils of subjects with localized aggressive periodontitis exhibit impaired chemotaxis that may exacerbate the disease (8,9), although recent studies suggest that localized aggressive periodontitis polymorphonuclear neutrophils are 'hyperfunctional' in other respects (10). In addition, localized aggressive periodontitis monocytes display a unique phenotype that promotes immunoglobulin G2 production (11) and predisposes these cells to differentiate into dendritic cells (12). Together, these data suggest that the unique host response of subjects with localized aggressive periodontitis can be traced to the distinct phenotypes of their myeloid cells.

Differential synthesis and degradation of immune response mediators by myeloid cells could impact the progression and severity of localized aggressive periodontitis. One such biologically relevant mediator is platelet-activating factor (1-O-alkyl-2acetyl-sn-glycero-3-phosphocholine), a pro-inflammatory phospholipid. Platelet-activating factor is synthesized primarily by monocytes, polymorphonuclear neutrophils and endothelial cells through a remodeling pathway (13-15), and targets a variety of cells through its G-protein-coupled receptor (16). Upon ligand binding, the plateletactivating factor receptor mediates diverse biological processes, ranging from cell adhesion, differentiation, angiogenesis and neurotransmission, to effects on reproductive and cardiac biology, allergy and sepsis, among others. Platelet-activating factor levels in gingival crevicular fluid and saliva correlate directly with the severity of periodontal disease and decline with successful treatment of disease (17–21). Platelet-activating factor induces the inflammatory response in subjects with localized aggressive periodontitis by activating monocytes to produce the T helper-1-associated cytokines, interleukin-12 and interleukin-18 (22). In addition, platelet-activating factor acts directly on localized aggressive periodontitis lymphocytes to elicit interferon- γ , the prototypic, proinflammatory T helper 1 cytokine (22). As inflammation plays a major role in the pathogenesis of periodontal disease, these studies suggest that plateletactivating factor exacerbates disease. However, platelet-activating factor also elicits the cytokines associated with protective immunoglobulin G2 antibody responses that correlate with reduced disease severity (23). Thus, the biology of platelet-activating factor is complex and this mediator can mediate both protective and destructive effects.

Both catabolism and synthesis contribute to the overall level of plateletactivating factor, and we hypothesize that the increased level of plateletactivating factor observed in subjects with localized aggressive periodontitis is secondary to both reduced catabolism by the catabolic enzyme plateletactivating factor acetylhydrolase and increased synthesis by monocytes or polymorphonuclear neutrophils, the leukocytes that synthesize plateletactivating factor. In a previous study, we demonstrated decreased plateletactivating factor acetylhydrolase activity in localized aggressive periodontitis monocytes compared with monocytes of control subjects (24). The difference in platelet-activating factor acetylhydrolase activity correlated with the propensity of localized aggressive periodontitis monocytes to differentiate into dendritic cells, which have low levels of platelet-activating factor acetylhydrolase (12,24). The current study was intended to elucidate the role of synthesis in determining plateletactivating factor levels in subjects with localized aggressive periodontitis and to assess the contributions of monocytes and polymorphonuclear neutrophils to the elevated plateletactivating factor levels observed in subjects with localized aggressive periodontitis.

Material and methods

Human subjects

Human studies were performed in compliance with all relevant federal guidelines and the institutional policies of Virginia Commonwealth University. All subjects were obtained by the Clinical Research Center for Periodontal Disease, School of Dentistry, Virginia Commonwealth University (Richmond, VA, USA). Patients with localized aggressive periodontitis had localized patterns of severe periodontal destruction limited to the first molars and incisors, with involvement of up to two additional teeth. Subjects with generalized aggressive periodontitis had at least eight teeth affected by \geq 5 mm of attachment loss, at least three of which were not molars or incisors. Both subsets of subjects with aggressive periodontitis had evidence of disease onset before 35 years of age. Subjects with chronic periodontitis had at least 2 mm of attachment loss on more than one tooth, disease onset after age 30 years and no evidence of rapid loss of attachment. The nonperiodontitis (periodontally healthy) control subjects were age and racematched to the periodontitis subjects. Periodontally healthy subjects had no evidence of attachment loss, except for recession on the buccal surface of anterior teeth at no more than one site, and no pockets with a depth greater than 3 mm. All subjects were African-American and nonsmokers.

Isolation of monocytes from peripheral blood

Thirty millilitres of heparinized human peripheral blood was added to 20 ml of RPMI, and 25 ml of that suspension was layered over 20 ml of lymphocyte separation media (MP Biomedicals, Aurora, OH, USA), and centrifuged at 400 g for 30 min. Peripheral blood leukocytes were then collected from the interface and washed three times with sterile RPMI. Adherent monocytes were obtained by culturing 10^7 peripheral blood leukocytes per well in 1 ml of RPMI on plastic six-well plates for 1 h at 37° C, in 5% CO₂. After incubation, the nonadherent cells were removed by extensive washing of the adherent monolayer with RPMI. Adherent monocytes were metabolically labeled and platelet-activating factor synthesis was quantified as described below.

Isolation of polymorphonuclear neutrophils from peripheral blood

Polymorphonuclear neutrophils were separated by a method described by Scott-Zaki et al. (25). Briefly, 30 ml of peripheral blood was collected in heparinized syringes. Polymorphonuclear neutrophils were separated by layering the blood over a gradient of Ficoll-Hypaque at densities of 1.077 and 1.119 (Sigma-Aldrich, St Louis, MO, USA). The tubes were then centrifuged at 250 g for 10 min, rotated 180° and centrifuged for an additional 10 min. The polymorphonuclear neutrophils, which sedimented at the 1.119 interface, were collected without disturbing the red blood cell pellet layer directly below. Polymorphonuclear neutrophils were washed with Hanks' balanced salt solution containing 0.1% bovine serum albumin and 0.01 M EDTA and further separated over a second identical Ficoll-Hypaque gradient. The polymorphonuclear neutrophils were collected from the 1.119 interface, washed with Hanks' balanced salt solution containing 0.1% bovine serum albumin and 0.01 м EDTA, then used for the platelet-activating factor synthesis assay.

Platelet-activating factor synthesis assay

Platelet-activating factor synthesis by polymorphonuclear neutrophils and monocytes was quantified with a metabolic labeling approach, as previously described (26). Ten million cells (polymorphonuclear neutrophils or monocytes) were incubated, for 10 min at 37°C, in Hanks' balanced salt solution containing 25 μ Ci/ml [³H]acetic acid (3.10 Ci/mmol; Perkin Elmer, Boston, MA, USA) and 25 mg/ml of fatty acid-free bovine serum albumin. After 10 min of prelabelling with [³H]acetic acid, 15 μ M calcium ionophore A21837 (Sigma/Aldrich) or vehicle (dimethylsulfoxide) was added and the incubation continued for an additional 50 min. After the incubation, cells were harvested and washed with Hanks' balanced salt solution to remove excess radiolabel. Lipids were extracted using the method of Bligh & Dyer (27) and separated by thin-layer chromatography using a previously described method (24). Platelet-activating factor was identified based on comigration with authentic plateletactivating factor (Avanti, Alabaster, AL, USA). The platelet-activating factor band was scraped from the thinlayer chromatography plate, and radiolabeled platelet-activating factor was quantified by scintillation counting and normalized to total acetate uptake. This assay measures total platelet-activating factor accumulation and does not distinguish between alkyl-plateletactivating factor and acyl-platelet-activating factor.

Statistical methods

Normalized platelet-activating factor synthesis (decays per minute plateletactivating factor + decays per minute acetate incorporated) was assessed by replicate determinations within each subject under two treatment conditions: vehicle (dimethylsulfoxide, resting cells); or 15 µм calcium ionophore A23187 (activated cells). Normalized platelet-activating factor synthesis proportions were estimated by the log difference of decays per minute in platelet-activating factor and total decays per minute incorporated, yielding a ratio of the geometric means. The repeated measures were taken into account by a mixed-model repeatedmeasures analysis of variance using the SAS MIXED procedure. The log-difference of the results were then summarized by back-transforming the results into proportions or ratios for interpretation.

Results

We hypothesized that increased synthesis would contribute to elevated platelet-activating factor levels in subjects with localized aggressive

periodontitis. To address this, we quantified platelet-activating factor synthesis by monocytes and polymorphonuclear neutrophils, two cell populations that are known to be atypical in subjects with localized aggressive periodontitis and are closely associated with platelet-activating factor synthesis. Monocytes and polymorphonuclear neutrophils were isolated from age- and race-matched subjects who were periodontally healthy or had localized aggressive periodontitis. The cells were metabolically labeled with [³H]acetate. incubated with vehicle (dimethylsulfoxide) or calcium ionophore A23187, subjected to lipid extraction and separation by thin-layer chromatography, and radiolabel incorporation in platelet-activating factor synthesis was quantified. The metabolic label was readily incorporated into monocytes and polymorphonuclear neutrophils of both subject groups. Although there was considerable subject-to-subject variability, mean acetate incorporation was not statistically different (p > 0.05)between resting monocytes (Fig. 1A) or polymorphonuclear neutrophils (Fig. 1B) from both subject groups. Stimulation with calcium ionophore A23187 did not alter acetate incorporation in either subject group or cell type (data not shown). To account for subject-to-subject variability in acetate uptake, platelet-activating factor synthesis data are expressed as the ratio of decays per minute in platelet-activating factor to decays per minute acetate uptake (normalized plateletactivating factor synthesis) throughout this report.

As shown in Table 1, vehicle-treated (resting) localized aggressive periodontitis and periodontally healthy monocytes synthesized similar amounts of platelet-activating factor. Similar results were obtained with monocytes from two additional subject groups: chronic periodonititis (chronic periodontitis); and generalized aggressive periodontitis (data not shown). In contrast, platelet-activating factor synthesis was significantly higher in resting localized aggressive periodontitis polymorphonuclear neutrophils than in periodontally healthy polymorphonuclear neutrophils (Table 2).



Fig. 1. Acetate incorporation by monocytes and polymorphonuclear neutrophils. Monocytes and polymorphonuclear neutrophils were isolated from the peripheral blood of periodontally healthy subjects and from subjects with localized aggressive periodontitis. The cells were metabolically labeled with 25 μ Ci/ml [³H]acetate for 60 min, after which an aliquot was harvested and scintillation counting performed to quantify acetate incorporation by the cells. The data are expressed as mean decays per minute acetate uptake (× 10⁻²). Error bars indicate the 95% confidence intervals. The data were collected from resting leukocytes. Acetate uptake by calcium ionophore A23187-stimulated leukocytes did not differ significantly from that of resting cells (p > 0.05, data not shown). (A) Acetate uptake by periodontally healthy (n = 5) and localized aggressive periodontitis (n = 8) monocytes. (B) Acetate uptake by periodontally healthy (n = 10) and localized aggressive periodontitis (n = 9) polymorphonuclear neutrophils. Acetate uptake did not differ significantly between localized aggressive periodontitis and periodontally healthy leukocytes (p > 0.05). dpm, decays per minute; LAgP, localized aggressive periodontitis; NP, nonperiodontitis (periodontally healthy).

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Table 1	Platelet_activating	tactor	synthesis	111	monocytes
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Treatment	Subject group	п	$Mean^a (\times 10^3)$	95% CI	
Vehicle	NP 5	5	3.3	1.6	6.8
	LAgP	8	2.8	1.6	4.9
IoA	NP	5	46.6	21.4	101.5
	LAgP	8	11.4	6.1	21.5 ^b

Monocytes were isolated from 'n' subjects, were metabolically labeled with [³H]acetate and were then incubated with vehicle (dimethylsulfoxide) or 15 μ M calcium ionophore A23817. Radiolabel incorporation into platelet-activating factor was quantified and normalized platelet-activating factor synthesis calculated as described in the Material and methods. ^aMean normalized platelet-activating factor synthesis × 10³ and 95% confidence intervals (95% CI).

^bStatistically different from periodontally healthy (p < 0.008).

IoA, calcium ionophore A23187; LAgP, localized aggressive periodontitis; NP, periodontally healthy.

Table 2. Platelet-activating factor synthesis in polymorphonuclear neutrophils

Treatment	Subject group	n 10	Mean ^a ($\times 10^3$)	95% CI	
Vehicle			0.1	0.1	0.2
	LAgP	9	0.3	0.2	0.7 ^b
IoA	NP	10	4.6	2.5	8.5
	LAgP	9	18.7	8.7	40.4 ^b

Polymorphonuclear neutrophils were isolated from '*n*' subjects, metabolically labeled with $[^{3}H]$ acetate and then incubated with vehicle (dimethylsulfoxide) or 15 μ M calcium ionophore A23817. Radiolabel incorporation into platelet-activating factor was quantified and normalized platelet-activating factor synthesis calculated as described in the Material and methods.

^aMean normalized platelet-activating factor synthesis \times 10³ and 95% confidence intervals (95% CI).

^bStatistically different from periodontally healthy (p < 0.02).

IoA, calcium ionophore A23187; LAgP, localized aggressive periodontitis; NP, periodontally healthy.

The 95% confidence intervals in Tables 1 and 2 were determined with a model that assumes equal variance in the localized aggressive periodontitis and periodontally healthy data sets. However, an analysis of the individual determinations suggests that variation within the localized aggressive periodontitis monocyte data set is considerably larger than that of the periodontally healthy data set (Fig 2, p < 0.03). The resting localized aggressive periodontitis and periodontally healthy polymorphonuclear neutrophils data sets exhibited similar degrees of variance (data not shown).

Activated leukocytes are major sources of platelet-activating factor (13 - 15).Platelet-activating factor production is induced by pro-inflammatory molecules, such as lipopolysaccharide (28,29) and monocyte chemoattractant protein-1, and we initially used these as stimuli for platelet-activating factor synthesis. However, subject-to-subject variation in the responses to these stimuli often exceeded differences between the diagnostic groups (data not shown), possibly caused by differences in expression of the receptors for these stimuli. Therefore, we chose a more potent, receptor-independent stimulus - calcium ionophore A23187 (15,26,30) - to compare platelet-activating factor synthesis in activated localized aggressive periodontitis and periodontally healthy leukocytes. As expected, calcium ionophore A23187 stimulation increased platelet-activating factor synthesis by leukocytes from both localized aggressive periodontitis and periodontally healthy subjects, with robust responses observed in both populations. Like resting localized aggressive periodontitis polymorphonuclear neutrophils, calcium ionophore A23187-activated localized aggressive periodontitis polymorphonuclear neutrophils synthesized more platelet-activating factor than did periodontally healthy polymorphonuclear neutrophils (Table 2, p < 0.02). Unexpectedly, localized aggressive periodontitis monocytes made a very modest response to calcium ionophore A23187 (Table 1), such that platelet-activating factor synthesis in this group was approximately fourfold lower than in calcium ionophore A23187-activated periodontally healthy monocytes. These data indicated that localized aggressive periodontitis monocytes synthesized less platelet-activating factor than did activated periodontally healthy monocytes. To assess, more directly, the responses of localized aggressive periodontitis monocytes to calcium ionophore A23187, we expressed the data as fold induction of platelet-activating factor synthesis in response to calcium ionophore A23187 (Fig. 3A) and also quantified the responses of monocytes from subjects with generalized aggressive periodontitis and chronic periodontitis. Similar amounts of platelet-activating factor were produced by resting monocytes from all four subject groups (see the Fig. 3 legend). When stimulated with calcium

A23187, periodontally ionophore healthy, chronic periodontitis and generalized aggressive periodontitis monocytes made similar responses, as calcium ionophore A23187 induced platelet-activating factor synthesis by \approx 12-fold. In marked contrast, localized aggressive periodontitis monocytes produced a more modest, approximately fourfold, response to calcium ionophore A23187 (p < 0.001). For comparison, fold induction of plateletactivating factor synthesis in polymorphonuclear neutrophils is shown in Fig. 3B. Resting periodontally healthy, chronic periodontitis, and generalized aggressive periodontitis polymorphonuclear neutrophils synthesized similar amounts of platelet-activating factor (see the Fig. 3 legend, p > 0.05) but resting localized aggressive periodontitis polymorphonuclear neutrophils synthesized more platelet-activating factor than did periodontally healthy polymorphonuclear neutrophils (Table 2), but not generalized aggressive periodontitis or chronic periodontitis polymorphonuclear neutrophils (data not shown, p > 0.05). Although the absolute amount of platelet-activating factor synthesized by localized aggressive periodontitis polymorphonuclear neutrophils was higher than that of periodontally healthy polymorphonuclear neutrophils (Table 2), the relative response to calcium ionophore A23187 (fold induction) was similar in all four groups of polymorphonuclear neutrophils (Fig. 3B, p > 0.05). Together, these data suggest that localized aggressive periodontitis monocytes have a unique phenotype, with respect to calcium signaling, that may impact on platelet-activating factor synthesis in activated cells.

Discussion

Other investigators have demonstrated that platelet-activating factor levels are higher in the tissues of subjects with localized aggressive periodontitis than in tissues of periodontally healthy subjects (17–21). Our data support the hypothesis that platelet-activating factor accumulation in localized aggressive periodontitis can be attributed to alterations in both its catabolism and



Fig. 2. Variance in platelet-activating factor synthesis in resting monocytes. Lipids were extracted from metabolically labeled resting monocytes from periodontally healthy subjects (n = 5) and from subjects with localized aggressive periodontitis (n = 8). Platelet-activating factor was separated from other lipids by thin-layer chromatography, the platelet-activating factor spots were identified and scraped from the thin-layer chromatography plates, and radiolabel incorporation was quantified by scintillation counting. Individual data are shown for each monocyte preparation studied, expressed as the mean normalized platelet-activating factor synthesis × 10³. Normalized platelet-activating factor synthesis is the ratio of decays per minute in platelet-activating factor to total decays per minute [³H]acetate incorporated by the cells. *Variance within the population of subjects with localized aggressive periodontitis was significantly different from the periodontally healthy population (p = 0.003). LAgP, localized aggressive periodontitis; NP, nonperiodontitis (periodontally healthy).

its synthesis, and that monocytes and polymorphonuclear neutrophils contribute differently to total plateletactivating factor levels. In a previous study (24), we reported that the activity of the catabolic phospholipase platelet-activating factor acetylhydrolase is lower in localized aggressive periodontitis monocytes than in periohealthy monocytes. As dontallv monocytes are the major source of platelet-activating factor acetylhydrolase (31), these data suggest that the unique phenotype of localized aggressive periodontitis monocytes contributes to platelet-activating factor levels through reduced catabolism. In the current study, we demonstrate that resting and activated localized aggressive periodontitis polymorphonuclear neutrophils synthesize more plateletactivating factor than do periodontally healthy polymorphonuclear neutroph-(Table 2). ils This observation indicates that polymorphonuclear neutrophils may contribute to the high levels of platelet-activating factor in the tissues of subjects with localized

aggressive periodontitis through increased anabolism. Thus, synthesis and degradation are likely to play important roles in platelet-activating factor accumulation in the tissues of localized aggressive periodontitis subjects, and monocytes and polymorphonuclear neutrophils differ in their contributions to platelet-activating factor accumulation.

In resting monocytes, normalized platelet-activating factor synthesis was similar in the localized aggressive periodontitis and periodontally healthy subject groups (Table 1). However, there was significantly greater variance in platelet-activating factor synthesis in the localized aggressive periodontitis subject group than in the periodontally healthy subject group (Fig. 2). At present, we are not certain why the localized aggressive periodontitis subject group exhibited greater variance. Platelet-activating factor is a proinflammatory lipid mediator that is produced when monocytes encounter microbial pathogens (16,32). Thus, the high levels of platelet-activating factor synthesis by the monocytes of some subjects with localized aggressive periodontitis could be episodic and a consequence of exacerbated disease. Additional experiments are necessary to test this hypothesis.

Resting localized aggressive periodontitis polymorphonuclear neutrophils synthesized more platelet-activating factor than did periodontally healthy polymorphonuclear neutrophils (Table 2), suggesting that polymorphonuclear neutrophils contribute to the high levels of platelet-activating factor, observed in subjects with localized aggressive periodontitis, through anabolism. The calcium ionophore, A23187, has previously been shown to platelet-activating induce factor (15,26,30) and was used to compare platelet-activating factor synthesis in activated periodontally healthy and localized aggressive periodontitis leukocytes. Although calcium ionophore A23187 induced platelet-activating factor synthesis in both monocytes and polymorphonuclear neutrophils from both subjects with localized aggressive periodontitis and periodontally healthy subjects, the degree of response varied between the groups. While calcium ionophore A23187 stimulated plateletactivating factor synthesis by more than 10-fold in periodontally healthy monocytes, localized aggressive periodontitis monocytes gave a more modest (approximately fourfold) response (Table 1, Fig. 3A). This observation is reminiscent of previous reports of aberrant calcium responses in localized aggressive periodontitis polymorphonuclear neutrophils that have been correlated with defective chemotaxis, reduced protein kinase C activation and reduced activity of a calcium influx factor (33-36). Thus, aberrant calcium signaling may also be a hallmark of localized aggressive periodontitis monocytes. The calcium ionophore A23187 responses of two additional subject groups - generalized aggressive periodontitis and chronic periodontitis were similar to those of periodontally healthy monocytes (Fig. 3A). These observations highlight the unique phenotype of the localized aggressive periodontitis monocyte with respect to lipid metabolism and calcium signaling.



Fig. 3. Localized aggressive periodontitis monocytes make modest platelet-activating factor responses to calcium ionophore A23187. Metabolically labeled monocytes from periodontally healthy subjects (n = 5), and from subjects with localized aggressive periodontitis (n = 8), generalized aggressive periodontitis (n = 5) and chronic periodontitis (n = 5) were stimulated with 15 µM calcium ionophore A23187. Lipids were extracted, radiolabel incorporation into platelet-activating factor was quantified, and normalized platelet-activating factor synthesis was calculated as described in the legend of Fig. 2. The data are expressed as fold induction of platelet-activating factor synthesis in response to calcium ionophore A23187, calculated as the ratio of normalized platelet-activating factor synthesis in calcium ionophore A23187-treated cells to normalized platelet-activating factor synthesis vehicletreated cells. The mean and 95% confidence intervals (error bars) are shown. (A) Fold induction of platelet-activating factor synthesis in calcium ionophore A23187-stimulated monocytes. Normalized platelet-activating factor production by resting monocytes was as follows [mean (95% confidence interval) $\times 10^3$]: periodontally healthy, 3.3 (1.6, 6.8); localized aggressive periodontitis, 2.8 (1.6, 4.9); generalized aggressive periodontitis, 2.0 (1.0, 4.1); and chronic periodontitis, 3.6 (1.7, 7.4). *The calcium ionophore A23187 responses of periodontally healthy, chronic periodontitis and generalized aggressive periodontitis monocytes were more robust than those of localized aggressive periodontitis monocytes (p = 0.0001). (B) Fold induction of platelet-activating factor synthesis in calcium ionophore A23187-stimulated polymorphonuclear neutrophils. Normalized platelet-activating factor synthesis by resting polymorphonuclear neutrophils was as follows [mean (95% confidence intervals) $\times 10^3$]: periodontally healthy, 0.08 (0.05, 0.16); localized aggressive periodontitis, 0.32 (0.15, 0.71); generalized aggressive periodontitis, 0.22 (0.09, 0.53); and chronic periodontitis, 0.18 (0.06, 0.54). The calcium ionophore A23187 responses of periodontally healthy, chronic periodontitis, generalized aggressive periodontitis and localized aggressive periodontitis polymorphonuclear neutrophils were not significantly different (p > 0.05).

The modest response of localized aggressive periodontitis monocytes to calcium ionophore was somewhat unexpected as these cells are known to be hyper-responsive to at least one other stimulus, lipopolysaccharide (37,38). As calcium is not involved in lipopolysaccharide signaling, these observations support the hypothesis that localized aggressive periodontitis monocytes may have selective alterations in calcium signaling and not more general defects in signal transduction. Alternatively, calcium signaling, in response to physiological stimuli, may be intact in localized aggressive periodontitis monocytes, and the blunted calcium ionophore A23187 response of localized aggressive periodontitis monocytes may be specific to this nonphysiological stimulus. At present, the specific mechanisms for blunted responses to calcium ionophore A23187 by localized aggressive periodontitis monocytes are uncertain.

It is of interest that calcium ionophore A23187 signaling appears to be intact in monocytes from subjects with generalized aggressive periodontitis, a closely related disorder. Localized aggressive periodontitis and generalized aggressive periodontitis tend to appear in the same families and are sometimes classified as alternate forms of the same disease (39). However, there is a precedence for differential immune responses in localized aggressive periodontitis and generalized aggressive periodontitis subjects. For example, although lipopolysaccharide triggers the same robust interleukin-1ß responses in localized aggressive periodontitis and generalized aggressive periodontitis monocytes, the localized aggressive periodontitis cells produce considerably more prostaglandin E₂ and tumor necrosis factor-a than do generalized aggressive periodontitis monocytes (37). Furthermore, while smoking suppresses circulating immunoglobulin G2 antibodies in periodontally healthy and generalized aggressive periodontitis subjects, immunoglobulin G2 levels are not affected in subjects with localized aggressive periodontitis who smoke (40). Together, these data underscore the unique biology of localized aggressive periodontitis and suggest that this disease may be separate and distinct from generalized aggressive periodontitis.

Previous reports indicate that localized aggressive periodontitis polymorphonuclear neutrophils also manifest aberrant calcium responses (10,33–36). Consistent with this, calcium ionophore A23187-stimulated localized aggressive periodontitis polymorphonuclear neutrophils produced more platelet-activating factor than did calcium ionophore A23187-stimulated periodontally healthy polymorphonu-

clear neutrophils (Table 2). However, the fold induction of platelet-activating factor synthesis in calcium ionophore A23187-stimulated localized aggressive periodontitis polymorphonuclear neutrophils was similar to that of periodontally healthy, chronic periodontitis and generalized aggressive periodontitis polymorphonuclear neutrophils (Fig. 3B). These observations suggest that the aberrant calcium responses of localized aggressive periodontitis polymorphonuclear neutrophils do not hinder calcium ionophore A23187-induced platelet-activating factor synthesis. The response to calcium ionophore A23187, a nonphysiological stimulus that induces exceptionally large increments in cytosolic calcium, may be so robust that it masks the more modest alterations in the calcium responses of localized aggressive periodontitis polymorphonuclear neutrophils. Alternatively, the calcium ionophore A23187 stimulus may compensate for defects in the calcium signaling of localized aggressive periodontitis polymorphonuclear neutrophils. Previous reports indicate that stimulus-dependent release of calcium from endoplasmic reticulum stores is intact in localized aggressive periodontitis polymorphonuclear neutrophils, but that the entry of extracellular calcium and/ or redistribution of intracellular calcium are aberrant (10,34). As a result, N-formyl-methionyl-leucyl-phenylalanine and interleukin 8-stimulated localized aggressive periodontitis polymorphonuclear neutrophils exhibit more robust increases in cytosolic calcium than do control polymorphonuclear neutrophils (10). It is possible that the same is true for calcium ionophore A23187-activated localized aggressive periodontitis polymorphonuclear neutrophils and that this results in increased platelet-activating factor synthesis. Further experiments are needed to delineate the differences in intracellular calcium stores and calcium signaling in localized aggressive periodontitis polymorphonuclear neutrophils and monocytes.

In addition to calcium, other signaling mechanisms could contribute to differential platelet-activating factor synthesis by localized aggressive periodontitis and periodontally healthy leukocytes. Other investigators (41,42) have demonstrated that diacylglycerol kinase- α expression is reduced in localized aggressive periodontitis polymorphonuclear neutrophils compared with periodontally healthy cells, resulting in increased diacylglycerol mass (41,43). As polymorphonuclear neutrophils synthesize platelet-activating factor through the remodeling pathway (18,44), this increase in diacylglycerol mass is not likely to contribute to platelet-activating factor synthesis directly, by acting as a substrate. However diacylglycerol accumulation could drive platelet-activating factor synthesis through the activation of protein kinase C, as other protein kinase C activators induce platelet-activating factor synthesis by polymorphonuclear neutrophils (26,45), and calcium ionophore A23187induced platelet-activating factor synthesis is suppressed by sphingosine, a protein kinase C inhibitor (45). Both calcium ionophore A23187and N-formyl-methionyl-leucyl-phenylalaninestimulated platelet-activating factor synthesis is suppressed by agents that elevate cAMP (46,47). As noted above, compared with periodontally healthy monocytes, localized aggressive periodontitis monocytes have an increased propensity to differentiate into dendritic cells (12). Interestingly, a recent study indicates that PDE1B2, a cyclic nucleotide phosphodiesterase, is expressed at lower levels in dendritic cells than in macrophages (48). These observations raise the possibility that cAMP levels could be elevated in localized aggressive periodontitis monocytes. Localized aggressive periodontitis monocytes produce high levels of prostaglandin E_2 (37). This mediator could bind its G-protein-coupled receptor and thereby activate adenylate cyclase, again resulting in higher levels of cAMP in localized aggressive periodontitis monocytes than in periodontally healthy monocytes. Through either mechanism, elevated cAMP in localized aggressive periodontitis monocytes could limit platelet-activating factor production by these cells. Finally, calcium ionophore A23187stimulated platelet-activating factor

synthesis is dependent on activation of the mitogen-activated protein kinases erk and p38 (49). Dendritic cells are known to be refractory to erk activation in response to certain stimuli (50). Given the propensity of localized aggressive periodontitis monocytes to differentiate into dendritic cells (12), these observations prompt the hypothesis that platelet-activating factor synthesis by calcium ionophore A23187-activated localized aggressive periodontitis monocytes is limited because these cells differentiate into dendritic cells that activate erk inefficiently. We are currently performing experiments to test all these hypotheses and more clearly delineate the mechanisms underscoring differential platelet-activating factor synthesis by localized aggressive periodontitis and periodontally healthy leukocytes.

Our studies add to a growing body of evidence that localized aggressive periodontitis leukocytes exhibit an unusual lipid metabolism that impacts on their ability to mount responses against oral pathogens. As noted above, diacylglycerol accumulates in localized aggressive periodontitis polymorphonuclear neutrophils owing to reduced activity and expression of diacylglycerol kinase- α (41,43). It has recently been suggested that the accumulation of diacylglycerol may render localized aggressive periodontitis polymorphonuclear neutrophils 'hyperfunctional' and thereby exacerbate inflammatory responses associated with the disease (51). Localized aggressive periodontitis polymorphonuclear neutrophils are known to synthesize large amounts of leukotriene B4 and lipoxin A_4 , bioactive lipids that have pro- and anti-inflammatory activities, respectively (41,51). Interestingly, a recent report indicates that polymorphonuclear neutrophils from subjects with localized aggressive periodontitis are hyporesponsive to the antiinflammatory actions of a lipoxin analog (52). Prostaglandin E_2 levels are high in localized aggressive periodontitis and have been suggested as a measure of disease severity (53-55). Although localized aggressive periodontitis monocytes have historically been implicated as the source of this bioactive lipid (37), more recent reports suggest that activated polymorphonuclear neutrophils may also be a rich source of prostaglandin E₂ (56). Prostaglandin E₂ is associated both with exacerbation of disease and the induction of protective immunoglobulin G2 antibodies that lessen disease severity (23,57). The biology of platelet-activating factor is quite similar as it promotes both inflammation and immunoglobulin G2 antibodies (16,22,23). Our next challenge is to distinguish the molecular mechanisms and intracellular signaling pathways associated with the protective vs. the pro-inflammatory responses of these bioactive lipids. Increased understanding of lipid metabolism and signaling in localized aggressive periodontitis myeloid cells may allow us to harness this unique cell biology to devise strategies to control the disease.

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