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Changes in Inflammatory Mediators in Experimental Periodontitis in the Rhesus Monkey

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Ligature-induced periodontitis was monitored for 6 months in eight *Macaca mulatta* monkeys to examine clinical status, radiographic bone level, and crevicular fluid (CF) levels of prostaglandin E_2 (PGE_2), thromboxane B_2 (TxB_2), interleukin-1β (IL-1β), tumor necrosis factor alpha, and leukotriene B_4 (LTB_4). A split-mouth design was used, with eight ligated teeth and eight contralateral nonligated teeth which develop soft-chow-promoted (spontaneous) disease. Ligated sites experienced an average attachment loss of 0.94 mm per site and linear bone loss of 0.88 mm per site, with spontaneous-periodontitis sites experiencing approximately half the loss of ligated sites. The CF mediator levels showed increased levels of PGE_2 and TxB_2 at the ligated sites, as compared with the spontaneous sites, with no significant contralateral differences in the IL-1β or LTB_4 responses. The concentrations of LTB_4 in CF reached an early threefold peak over the baseline level at 1 month. By 2 months there was a statistically significant threefold elevation in CF-PGE_2 in the ligated sites and a twofold elevation in the spontaneous sites as compared to the baseline level ($P = 0.041$ and 0.008, respectively). The monocyte product IL-1β increased sharply at 2 months and returned to the baseline level by 6 months at both ligated and nonligated sites. Tumor necrosis factor alpha in CF was below the limit of detection at all sites throughout the experiment (i.e., <2 ng/ml). The selective elevation of both PGE_2 and TxB_2 in ligated sites, compared with levels in spontaneous sites, in the presence of similar levels of LTB_4 and IL-1β provides further evidence that these molecules regulate the magnitude of the tissue-destructive response in progressive periodontitis.

Experimental periodontitis in the nonhuman primate has proven to be a useful model for understanding the molecular basis of the inflammatory lesion (14, 15), the virulence properties of putative periodontal pathogens (7), and the potential efficacy of pharmacological agents (13, 14, 27) in human periodontal disease. The rhesus monkey develops periodontitis spontaneously in captivity with certain characteristics that are similar to the disease in humans in that it appears to be relatively slow in progressing and demonstrates increased severity with age (1, 3, 4, 11, 12, 20, 21). Experimental models which employ ligatures and/or soft chow to promote plaque retention accelerate disease progression. Ligature placement results in a rapid loss of about 1 mm of probing attachment per site in about 1 to 2 months which is followed by a parallel loss of bone by 3 to 6 months (14, 15). In these animals, disease progression occurs at nonligated sites but results in a slower attachment and bone loss pattern which requires about 1 year to reach 1 mm per site (14). It is not entirely clear exactly how these two disease models relate to human disease states. Longitudinal data on the natural progression of periodontitis in humans suggest that disease progression can occur either rapidly in episodes or more slowly in a chronic, almost linearly progressive manner (for a review, see reference 6). It seems reasonable to suggest that the differences in clinical presentation when these two types of progression are compared may be due to either a simple difference in chronological development or different mechanisms or sequences of cellular recruitment and activation. Clinically, the ligature model in the rhesus monkey mimics the more acute episodic loss that can occur in humans, and the soft-chow, or "spontaneous" periodontitis, model relates to the more chronic progressive lesion in humans. Therefore, the intent of this report is to provide data describing the chronological development of these two types of lesions and to contrast some of the underlying molecular inflammatory events which occur in these two forms of periodontal disease. We have focused our studies on relating the changes in clinical disease expression to the changes in the crevicular fluid (CF) levels of prostaglandin E_2 (PGE_2), thromboxane B_2 (TxB_2), leukotriene B_4 (LTB_4), interleukin-1β (IL-1β), and tumor necrosis factor alpha (TNF-α). Our previous studies have demonstrated CF increases in both PGE_2 and TxB_2 in both forms of this disease model (15–18). Our intent is to confirm these findings and to relate these changes temporally to these other important inflammatory mediators. LTB_4 is a neutrophil product that is vasoactive and plays a significant role in neutrophil and monocyte recruitment and activation (for a review, see reference 2). We have previously described elevated LTB_4 levels in adult and juvenile periodontitis (17) as well as in gingivitis (6). In this report we establish the relationship between LTB_4 elevation and longitudinal disease progression. IL-1β and TNF-α are principally products of activated monocytes which are critical for lymphocytic clonal expansion and inflammatory cell activation, and both are potent stimulators of osteoclastic bone resorption (for a review, see reference 8). Data presented reveal insight into the sequence of cellular activation events which occur in the rhesus monkey and suggest underlying mechanisms of pathogenesis for progressing lesions. We provide data in

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support of an orchestrated, sequential model for cytokine- and lipid mediator-directed periodontal destruction.

MATERIALS AND METHODS

Experimental animals. Eight rhesus monkeys (*Macaca mulatta*) with clinical signs of periodontitis but showing no systemic illness were selected for this study. Animals were housed at Yerkes Regional Primate Research Center as described previously (1). In addition to their use in the present study, this group of animals served as a control group for a study of a topical intraoral gel formulation. The placebo gel did not apparently affect plaque accumulation or the rate of disease progression, as compared with results of our previous studies (14, 18). Prior to the study there was a 2-week chair-training period for the monkeys. Each day the animals were removed from their cages with a rigid collar with an interlocking pole (Primate Products). The animals were guided into a passive restraining chair with an interlocking neck plate (Primate Products) that isolated and restrained the monkey's head. Any monkey who resisted daily handling or who failed to learn the chairing routine with a minimum of apparent stress was excluded.

Disease model. The disease model employed a split-mouth design. A Cavitron prophylaxis was completed on the left side of the mouth 2 weeks before the study began. At the beginning of the study, the animals were placed on a diet of water-softened Purina monkey chow supplemented with soft fruit. On the left side of the mouth, teeth 9, 12, 13, 14, 19, 20, 21, and 24 were monitored for the development of soft-chow-promoted periodontal disease. These teeth were not ligated and are designated spontaneous sites in this report. Ligature-induced periodontitis was started on the right side of the mouth by ligating eight test teeth (teeth 3, 4, 5, 8, 25, 28, 29, and 30) at the cementoenamel junction with 3-0 silk. Daily log sheets were used to record any lost ligatures, which were replaced at 2-week intervals.

Clinical examinations. On examination days, five separate stations were set up and the monkeys were rotated through each station to minimize the amount of time that each monkey was sedated. At the first station, animals were anesthetized with 4 to 5 mg of Telezol (Aveco Company, Inc., Fort Dodge, Iowa) per kg, administered intramuscularly, which we found to be superior to ketamine since it reduced salivation and minimized seizures. A veterinarian or veterinarian's assistant anesthetized, weighed, and collected a blood sample from each animal and subsequently followed that animal through all stations to monitor anesthesia and provide supplement, if needed. Each station had one operator who collected all samples, clinical indices, or X-rays for all animals to eliminate intraoperator variability in data collection and clinical assessments. This same operator was present at all examination visits. The animals were moved sequentially through the stations—anesthesia, radiographs, CF sampling, clinical indices, and ligature placement—prior to returning to the recovery area. Thus, each animal was anesthetized for a total of about 1 h for each exam. For each clinical index three sites, the mesiobuccal, buccal, and distobuccal, were checked on each tooth by evaluation criteria previously described (14, 15). Briefly, gingival erythema was evaluated with an index of 0 to 4, with 0 to 3 indicating none, mild, moderate, and severe, respectively; a score of 4 was given if frank ulceration or necrosis was present. Bleeding on probing (using a controlled-force Florida probe [25 g]) was recorded as either present or absent at each site. Pocket depths and cementoenamel junction levels were recorded to the nearest 0.1 mm with the Florida probe, using the pocket measuring handpiece to enable Ramfjord attachment level calculations. Two weeks before the study began, all clinical measurements for all animals were collected. At the start of the study, these parameters were measured again. This enabled the comparison of all clinical measurements obtained on the right side of the mouth, which did not have a prophylaxis, at two time points to provide estimates of the variability of repeated measures.

Radiographic analyses. Standardized radiographs were taken as described previously, with a constant focal distance of 60 in. (ca. 152 cm) from the film (9). The X-ray unit was a Tokya Emix Corporation Model D, TR-20. Customized Rinn bite blocks and high-speed Type E Kodak film were used. The monkey's head was held by a head restraint to prevent any movement of the head and to standardize the geometry and the distance between the X-ray unit and the teeth. Mandibular molar and premolar X-rays were taken on the right and left sides for each monkey at day zero, 3 months, and 6 months.

Radiographs were digitized as previously described (9) with a video camera coupled to an analog-to-digital converter capable of storing an entire video frame in solid-state memory in one frame time (0.05 s). The frame grabber (PCVISION PLUS; Imaging Technologies, Woburn, Mass.) is capable of storing two image frames, with a spatial resolution of 512 by 512 pixels and a 256-level gray scale, by using a microcomputer (Compaq 386/20) as the central processing unit. Since slight variations in film processing or voltage to the X-ray tube may result in differences in contrast in the resultant films, the nonparametric gamma correction algorithm of Ruttimann (22) was used to correct for differences in contrast between the pretreatment and posttreatment radiographs. If necessary, planar geometric discrepancies were corrected by applying an aphine warp algorithm previously described (26). The distance between the cementoenamel junction and the crest of the alveolar bone was determined for mesial and distal root surfaces according to the method described by Jeffcoat et al. (27). The measurement was corrected for the magnification of the radiograph in the image processing system. Millimeters of bone loss at each site was determined for both ligated and spontaneous sites at day zero, 3 months, and 6 months. The millimeters of loss was subtracted from the loss at day zero to determine the amount of bone lost during the study. Previous studies have shown the error of this method in estimating bone loss to be less than 0.08 mm on the original radiograph (27). All radiographs were digitized and analyzed blindly. Each radiograph for digitized analysis was identified by number only; thus each bone height determination was made totally independently with no before-versus-after bias.

Biochemical analyses. CF samples were used for the determination of PGE_2, TxB_2, LTB_4, and IL-1β. The collection, storage, and analyses of these samples have been described previously (6, 15). Briefly, CF samples were collected and the volume was measured with a Model 6000 Periotron (Harco Electronics Ltd., Canada). Three CF samples were taken from each side of the mouth for each mediator. Site pairing was maintained for each mediator at all time points. The PGE_2 and TxB_2 were assayed by radioimmunoassay (RIA) as previously described (15). Standard curves and internal control samples from two sets of samples indicated technical RIA problems. These two datum points (PGE_2 at 1 month and TxB_2 at 2 months) were omitted from the data analysis and the figures. LTB_4 was assayed by a slight modification of the same RIA with immunoreagents supplied

by Advanced Magnetics. IL-1β and TNF-α levels were quantitated by using the Cistron enzyme-linked immunosorbent assay (ELISA) kits, graciously provided by Cistron. The IL-1β assay uses human IL-1β as a standard, a plate-bound, mouse anti-human IL-1β monoclonal antibody, and an antihuman IL-1β rabbit polyclonal antiserum. We had previously found these human IL-1β and TNF-α Cistron reagents to cross-react with rhesus IL-1β and TNF-α sufficiently to theoretically permit detection in rhesus CF. These experiments were performed on rhesus tissue samples (data not shown). However, since TNF-α could not be detected within the CF by using Cistron reagents, we also tested Medgenix TNF-α reagents with similar results, thereby confirming that the levels of TNF-α were below our detection level of 2 ng/ml.

Computations and statistics. After the biochemical analyses were completed, an interactive computerized data link system for fitting standard curves and for computation of unknowns was used. All clinical data were stored, sorted, and collated in spreadsheet files.

Data from multiple sites within each monkey were pooled to provide single-animal means ± standard error, with the monkey as the unit of observation. These values were then pooled to obtain group estimates. This approach was used for all clinical and biochemical data. By using a repeated-measures analysis of variance (ANOVA), comparisons were made for both disease model (ligated versus spontaneous) and time. When significant effects were found by repeated-measures ANOVA, pairwise comparisons were made by paired or nonpaired *t* tests. An alpha level of less than 0.05 was considered statistically significant.

RESULTS

General comments. During the first 3 months of the study most monkeys showed a weight loss of approximately 5% of their baseline body weight. This may have been due to the daily routine of chairing or to a flulike viral infection that spread throughout the primate colony, including our study animals. By the 3-month clinical evaluation, most of the weights had stabilized and remained stable for the remainder of the study. Periodontal disease progression in this model and changes in CF-PGE_2 were found to be essentially identical to those in our previous experiments which did not use either a placebo gel or chairing technique (14, 15).

Changes in clinical status. As expected, both the ligated and nonligated sites showed a significant time-dependent increase in redness and bleeding on probing. Figure 1 shows the average percentage of sites which demonstrated an increase in redness scores. This shows that ligated teeth had an average of 28.6% sites that showed an increase in redness score (of 1 or more units) at 1 month, as compared with the baseline level. By 2 months, 46.7% of the sites on ligated teeth showed an increase in redness score of 1 unit or more. The redness scores for both the ligated and spontaneous groups increased with time over the baseline levels at $P = 0.003$ and $P = 0.0016$, respectively. At 2 and 3 months, the ligated redness scores were significantly higher than the spontaneous redness scores ($P < 0.05$). The bleeding-on-probing data shown in Fig. 2 are expressed as the percentage of sites which bleed after being probed. The ligated sites increased from 50% at day zero to more than 70% by 2 months at $P < 0.05$. The spontaneous sites remained relatively stable throughout the 6-month study, with approximately 60% of the sites exhibiting bleeding. The cavitron prophylaxis 2 weeks before the study did not result in a significant reduction in the bleeding or redness baseline scores on the nonligated side of the mouth.

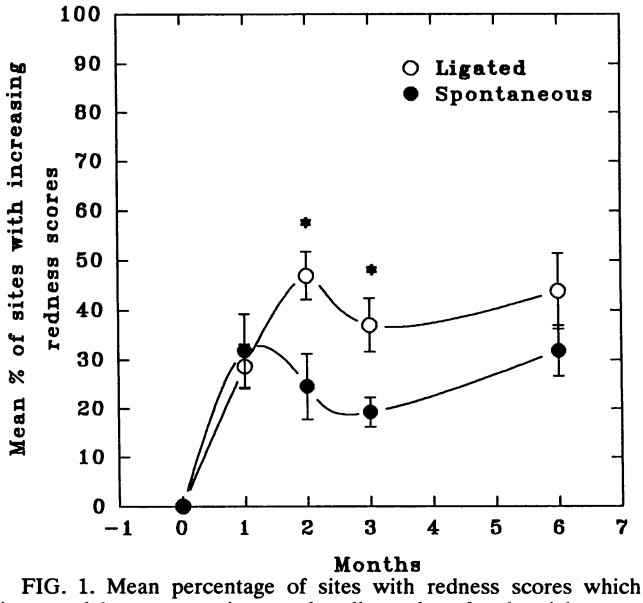

FIG. 1. Mean percentage of sites with redness scores which increased 1 or more units over baseline values for the eight monkeys. The mean values for the ligated and nonligated (spontaneous) sites are shown with standard error bars. Both ligated and spontaneous sites demonstrated an increase in redness scores over time at $P = 0.003$ and $P = 0.0016$, respectively. Asterisks indicate significant ($P \leq 0.05$) differences in spontaneous versus ligated sites.

The effects of ligation on attachment loss and bone loss are shown in Fig. 3 and 4, respectively. A significant loss of attachment of 0.38 mm per site for the ligated teeth was seen at 1 month; this increased to 0.71 mm per site at 2 months and 0.94 mm per site at 6 months. The nonligated teeth

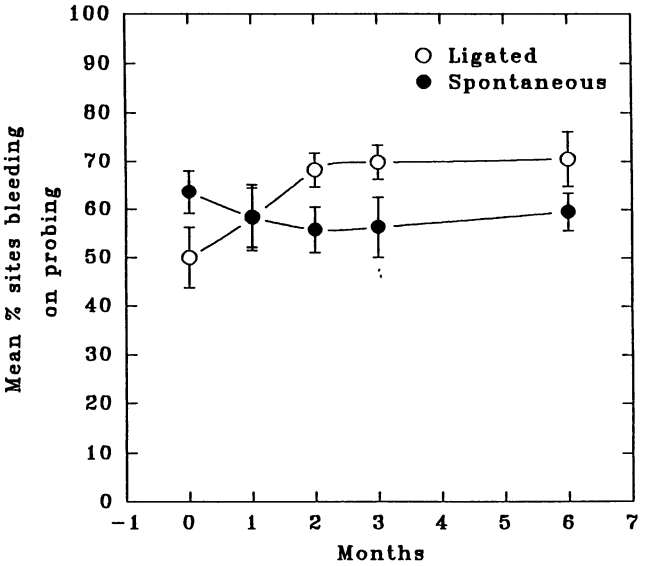

FIG. 2. Mean percentage of sites which exhibited bleeding upon probing with a constant-force probe (Florida probe, 25 g). Results for all monkeys are pooled. The ligated sites showed a significant increase in bleeding scores over time at $P < 0.05$, whereas the spontaneous sites did not ($P = 0.87$).

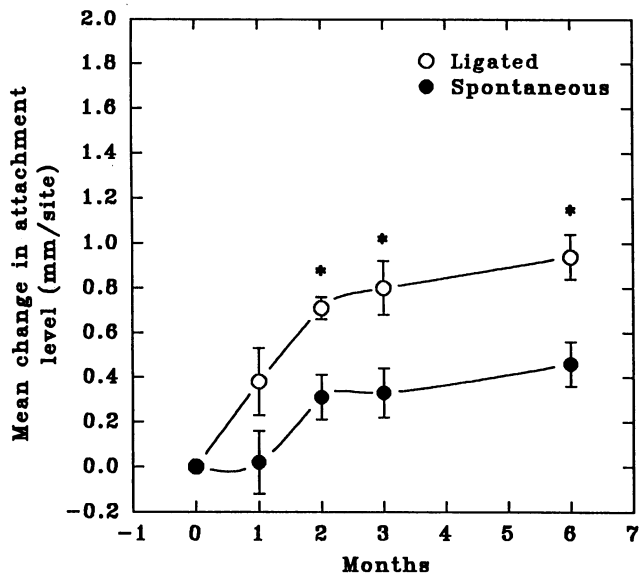

FIG. 3. Mean attachment loss in millimeters per site from the baseline level. Results for all animals are pooled. There was a significant increase in attachment loss over time on both sides, with greater loss occurring with the ligated teeth (P = 0.0001), as indicated by asterisks.

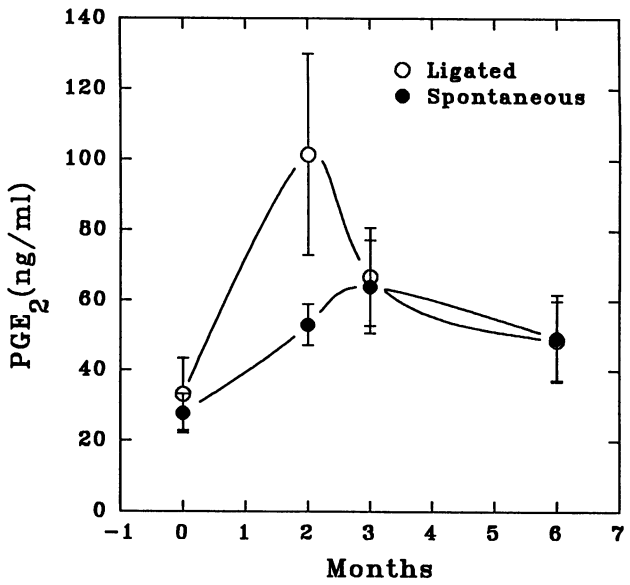

FIG. 5. CF-PGE_2 concentrations (in nanograms per milliliter) for ligated and spontaneous sites. The mean values for pooled sites are shown with standard error bars. CF-PGE_2 levels increased threefold and twofold over the baseline levels, respectively, for ligated and spontaneous sites. CF-PGE_2 concentrations at all sites decreased to near baseline levels by 6 months.

demonstrated a significant attachment loss by 2 months, reaching 0.46 mm per site by 6 months (P = 0.001). The ligated teeth lost significantly more attachment than the nonligated teeth at P = 0.0001. Both ligated and nonligated attachment loss appeared to increase from 0 to 2 months and plateau at 2 to 3 months. As seen in Fig. 4, there was a significant amount of bone loss on the ligated sites when either the 3-month or 6-month evaluation was compared to the baseline level at P = 0.045 and P = 0.016, respectively. A repeated-measures ANOVA showed no significant differences between the ligated and spontaneous bone losses. Compared with the baseline level, the amount of bone loss around the spontaneous teeth was significant at 3 months only.

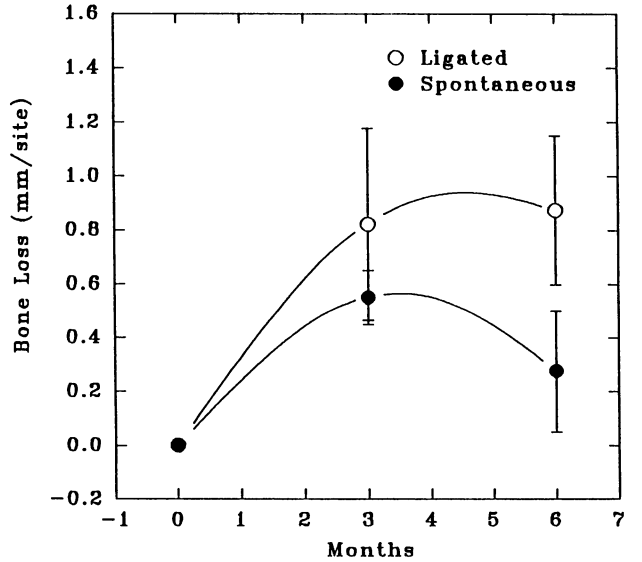

FIG. 4. Mean bone loss in millimeters per site (1 pixel = 0.1 mm) in ligated and nonligated mandibular molar and premolar sites. The ligated teeth demonstrated a significant loss of bone at 3 and 6 months at P = 0.016.

Changes in inflammatory mediators. At day zero there were no significant differences in the levels of PGE_2, TxB_2, LTB_4, or IL-1β in CF, comparing ligated with spontaneous disease. Figure 5 illustrates the changes in the PGE_2 concentrations in CF, showing all ligated sites pooled as the open circles and all spontaneous sites as the closed circles. By 2 months, there was a statistically significant threefold elevation in CF-PGE_2 in the ligated sites and a twofold elevation in the spontaneous sites, as compared with baseline levels (P = 0.041 and P = 0.008, respectively). There were no differences in the relative elevation in the ligated and spontaneous CF-PGE_2 levels at 2 and 3 months, and both declined towards the baseline level by 6 months. The time-dependent changes in CF-TxB_2 at ligated sites, as seen in Fig. 6, closely paralleled the CF changes in the PGE_2 levels, although the CF-TxB_2 appears to peak at 2 to 3 months, suggesting it lags behind the peak in CF-PGE_2 which occurs at approximately 1 to 2 months. The concentration of CF-TxB_2 significantly increased threefold in the ligated sites during the first 3 months of the study, corresponding to an increase from 33.7 ng/ml to 107.6 ng/ml, and decreased to the baseline level by 6 months. The mean CF-TxB_2 concentration on the spontaneous side appears to decrease below the baseline level; however the decrease was not statistically significant and was probably due to the large variability of CF-TxB_2 at day zero. The ligated sites had CF-TxB_2 levels significantly higher than those of spontaneous sites at 3 and 6 months at $P < 0.04$. Thus, ligation caused a tandem 3-month threefold rise in both cyclooxygenase (CO) products PGE_2 and TxB_2, whereas only PGE_2 was shown to increase in the nonligated sites. Significant attachment loss and bone loss occurred in both ligated and

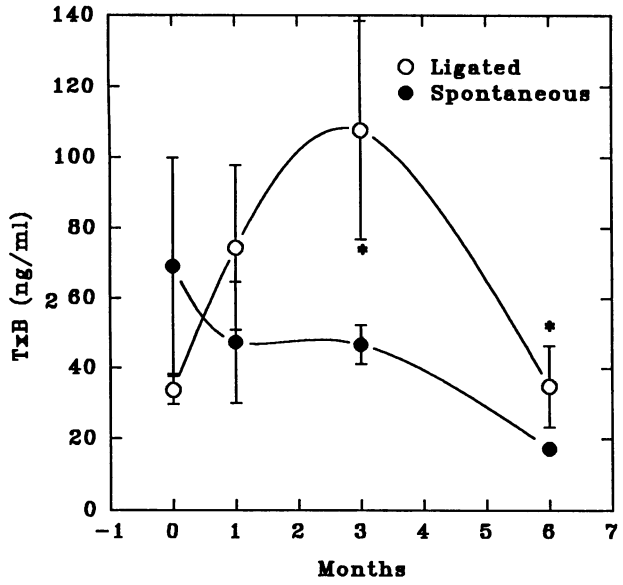

FIG. 6. CF-TxB_2 concentrations (in nanograms per milliliter) for ligated and spontaneous sites. Asterisks indicate significant ($P \leq 0.05$) differences in spontaneous versus ligated sites. The mean CF-TxB_2 values for ligated sites increased threefold during the first 3 months and returned to the baseline level by 6 months. The CF-TxB_2 concentrations for spontaneous sites did not change significantly from the baseline levels.

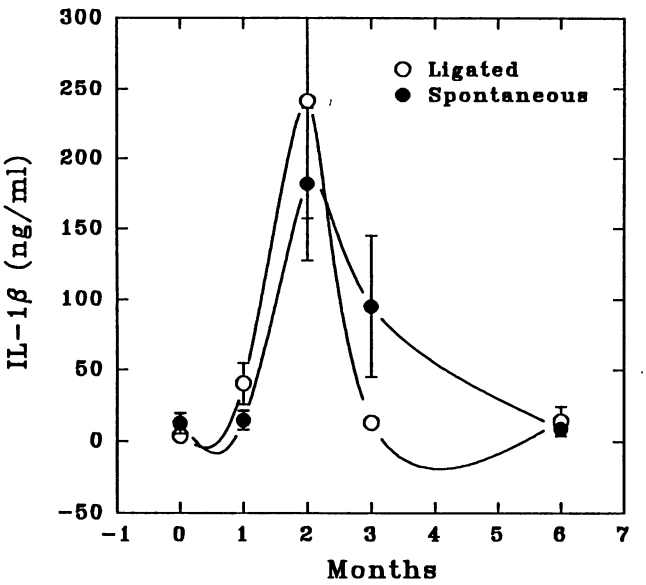

FIG. 8. CF-IL-1β concentrations (in nanograms per milliliter) for ligated and spontaneous sites. CF-IL-1β levels rose dramatically from near zero levels at day zero to peaks at 241.3 ± 41.8 ng/ml and 181.9 ± 31.7 ng/ml at 2 months for ligated and spontaneous sites, respectively. The significant rise for both groups was short-lived and returned to baseline levels at 6 months.

spontaneous sites, but PGE_2 was the only CO product to increase bilaterally. Thus, elevated CF-TxB_2 does not appear to be an absolute requisite for disease progression in this model.

The changes in the lipoxygenase (LO) product LTB_4 are shown in Fig. 7. In ligated sites there was a 3.6-fold, 1-month peak in LTB_4 levels compared with the baseline level. Repeated-measures ANOVA indicates that there is a time-dependent significant rise in LTB_4 levels in both ligated and spontaneous sites over the baseline level; levels for the two sides are not statistically different from each other. However, temporal changes of ligated LTB_4 levels with a 1-month peak, followed by a trough towards the baseline level, and a later 6-month rise are statistically significant fluctuations. In both the ligated and nonligated sites the increase in LTB_4 levels occurred early, resulting in a significant increase in the level in CF by 1 month. Thus, the rise in LO products, as measured by CF-LTB_4, preceded the observed increase in the CO products PGE_2 and TxB_2, which increased at 2 to 3 months (compare Fig. 7 to Fig. 5 and 6).

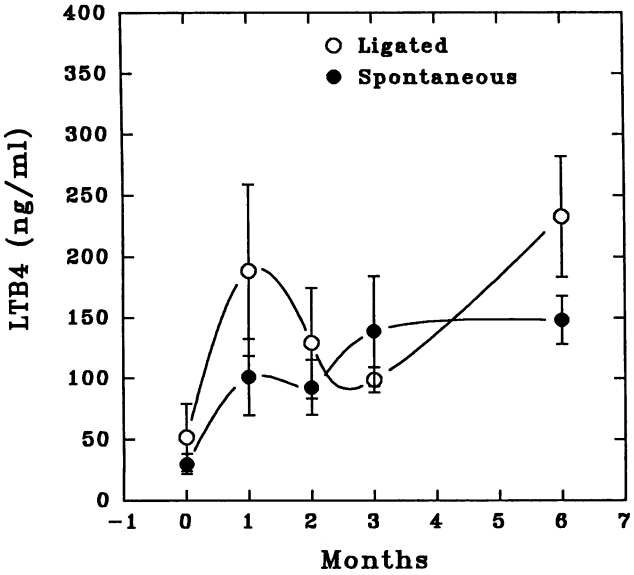

FIG. 7. CF-LTB_4 concentrations (in nanograms per milliliter) for ligated and spontaneous sites. Ligated sites experienced a rapid 3.6-fold increase at 1 month, followed by a decrease toward baseline at 2 and 3 months and another rise at 6 months. Increased CF-LTB_4 occurred in both groups by 1 month.

The changes in CF-IL-1β are shown in Fig. 8. At baseline the CF-IL-1β levels were low at both ligated and nonligated sites (3.9 and 12.5 ng/ml, respectively). At 2 months there was a dramatic rise in IL-1β levels, which reached peaks at 241.3 ± 41.8 ng/ml and 181.9 ± 31.7 ng/ml at ligated and spontaneous sites, respectively. This rise in IL-1β is significant at $P < 0.01$, as compared with the baseline levels. The short-term rise in IL-1β levels is noteworthy, as the levels remained low at 1 month in both ligated sites and spontaneous sites and levels for both sites returned to the baseline level by 6 months. Thus, the rise in IL-1β is rapid and short-lived in this model.

As stated previously, the levels of TNF-α in the CF were below detection limits (i.e., < 2.0 ng/ml) at all time points by two different ELISA systems.

DISCUSSION

In this study, ligated sites lost about twice as much attachment and bone as nonligated sites, in close agreement with our previous clinical findings in this model (15). Com-

paring inflammatory mediator levels in ligated versus nonligated sites demonstrates certain similarities and a few differences in the magnitude of the mediator responses. For example, IL-1β responses in ligated and nonligated sites are almost identical with regard to magnitude and timing (Fig. 8). LTB_4 responses are also similar, both showing an early rise at 1 month with a slightly elevated level at ligated sites, albeit insignificant statistically. Similarly, both CO products PGE_2 and TxB_2 have higher values at 2 to 3 months in the ligated sites than in the spontaneous sites. Thus, higher levels of these mediators may, in part, provide an explanation for the greater inflammation, attachment, and bone loss associated with ligature placement.

The only mediator which did not increase in either ligated or nonligated sites was TxB_2 in spontaneous-disease progression (Fig. 6). Ligation resulted in a marked increase in TxB_2 and PGE_2. PGE_2 also increased in nonligated sites without a concomitant rise in TxB_2, suggesting that these CO products are independently regulated. TxB_2 is predominantly produced by both platelets and monocytes (17). PGE_2 is synthesized predominantly by stimulated monocytes and fibroblasts. The concomitant elevation of TxB_2 and PGE_2, such as that observed in ligated sites, may reflect a concerted response by platelets, monocytes, and fibroblasts. However, the selective elevation of PGE_2 without a rise in TxB_2, as seen in spontaneous-disease progression, suggests that resident fibroblasts may be a major source of PGE_2 in this model. The bilateral elevation of PGE_2 remains a consistent factor for disease progression in both models. It also suggests that the enhanced CO activity due to ligature placement may be a result of increased monocyte and/or platelet activation. This concept is further supported when one examines the temporal development of the lesion.

In our previous experience with the experimental-periodontitis monkey model (15), we observed a three- to fivefold parallel increase in PGE_2 and TxB_2 as the dominant metabolite changes of the CO pathway. This rise in CF-PGE_2 and CF-TxB_2 reached a peak at 3 months, which was the earliest CF sampling period after initial ligature placement. The present data confirm these observations with regard to both the magnitude of the increase in PGE_2 and TxB_2 levels and the temporal relationship, placing the peak CO activity at 2 to 3 months. This finding is supplemented with new evidence for the early activation of the LO pathway preceding the burst of CO activity. This is manifested as a threefold increase in LTB_4 which peaks or plateaus by 1 month. We and others (2, 10) have evidence which indicates that LTB_4 is produced by activated neutrophils during terminal degranulation and cytolysis, presumably as a result of end-stage confrontation with bacteria. Other cells resident in the periodontium, such as keratinocytes, monocytes, fibroblasts, endothelial cells, myoblasts, platelets, mast cells, eosinophils, and bone cells, either do not possess LO or synthesize LO metabolites other than LTB_4 (2). For example, platelets possess LO, but they secrete 12-hydroxyeicosatetraenoic acid (12-HETE) and not LTB_4 (2). Among these cell types, only neutrophils appear to produce significant amounts of LTB_4, and these cells are also devoid of CO activity. These biochemical data suggest an early activation of neutrophils in this model, an observation that closely parallels the cellular histology of the ligature-induced periodontitis lesion which has been characterized as having an early accumulation of neutrophils (3, 4, 11, 12, 20, 21).

In the monkey model, the LTB_4 increase appears to peak or reach a plateau at 1 month, which is coincident with the attainment of at least half the maximum inflammatory response, as reflected in the changes in redness and bleeding scores in this and in our previous monkey studies (14, 15). This suggests that neutrophil activation and LTB_4 play a dominant role in initiating the superficial inflammatory response in this early phase of this progressive lesion. In other experiments, we examined the time course of CF-LTB_4 synthesis in experimental gingivitis in humans (6). In that study, we found a rapid rise in LTB_4 by 1 week which also preceded the rise in CO metabolites PGE_2 and TxB_2 which occurred between 3 to 4 weeks. This suggests that LTB_4 and neutrophil-mediated responses play an important role in the early development of gingival inflammation and precede changes in CO metabolites, IL-1β, or attachment loss and bone loss. The peak attachment loss and bone loss are more coincident with the later increase in PGE_2, TxB_2, and possibly IL-1β. This is consistent with our previous data which failed to convincingly link gingival inflammatory changes with CF-PGE_2 and TxB_2 but consistently associated high CF-PGE_2 and TxB_2 levels with attachment loss and bone loss in beagles (18), monkey (15), and humans (16).

In the monkey, the LTB_4 increase is followed by a period of increasing CO metabolites and IL-1β. We envision that this reflects a deeper penetration of bacterial challenge that has progressed beyond the neutrophil defenses and has stimulated the subjacent chronic macrophage infiltrate or the newly recruited, LTB_4-elicited monocytes. We would suggest that this monocytic activation represents the second phase of the lesion that would explain the later rise in levels of PGE_2 and TxB_2, which are the principal CO metabolites of these cell types. Deeper penetration of bacterial challenge, either antigenic or in the form of lipopolysaccharide (LPS; endotoxin) would also be expected to result in monocytic IL-1β release. This would explain the 2-month surge in IL-1β secretion (Fig. 8). The potential role of IL-1β in periodontal pathogenesis has been recently reviewed by Page et al. (19), and new data by Stashenko et al. (25) have demonstrated IL-1β to be present in active human lesions. IL-1β is principally a monocyte product that can be cosecreted, along with PGE_2 and TxB_2, as a result of exposure to either LPS or gamma interferon. Gamma interferon is a product of antigen-stimulated T helper cells (19). Thus, IL-1β increases may be a reflection of LPS or antigenic penetration. Although IL-1β has many proinflammatory properties (19), certain cellular activities are easily implicated when considering the pathogenesis of periodontitis. These include stimulation of collagenase secretion, bone resorption, and epithelial proliferation. Thus, IL-1β may play a key role in mediating the clinical signs of attachment loss, pocket formation, and radiographic bone loss. PGE_2 is known to synergistically augment the inflammatory and bone-destructive capacity of IL-1β (23). Thus, the selective elevation of PGE_2 in ligated versus nonligated sites in the presence of equal amounts of IL-1β may explain the increased bone and attachment loss that is induced by ligation.

The observation that TNF-α levels are below 2 ng/ml by our detection methods is in agreement with the recent data by Stashenko et al. (24) demonstrating the low levels of TNF-α within inflamed periodontal tissues relative to other cytokines such as IL-1β. We have previously observed that levels of LTB_4 (17) and PGE_2 (16) in tissue are 8- to 10-fold greater than those in the adjacent CF. Thus, the tissue TNF-α levels reported by Stashenko et al. (0.4 ng/ml) would be expected to be diluted below our detection threshold within the CF. These data further suggest that PGE_2 and IL-1β, rather than TNF-α, represent principal bone-resorbing molecules in this model.

In summary, these new biochemical data suggest a two-stage lesion. The first stage involves bacterial or neutrophil confrontation accompanied by LTB_4 release and clinical gingival inflammation. Thus, LTB_4 probably serves to recruit and activate neutrophils and monocytes. Near the end of this stage, there is also some mild activation of the CO pathway with slight elevations in PGE_2 and TxB_2. During the second stage of the lesion, the neutrophil defenses are overcome with full activation of the underlying monocytic cell lineage probably due to deeper LPS and antigenic penetration. This monocytic activation is accompanied by high levels of PGE_2, TxB_2, and IL-1β secretion with ensuing loss of attachment and bone.

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