The overexpression of cyclo-oxygenase-2 in chronic periodontitis

FAN ZHANG, M.D., Ph.D.; STEVEN P. ENGEBRETSON, D.M.D., M.S.; RAKHI S. MORTON, D.D.S., M.S.; PAUL F. CAVANAUGH JR., Ph.D.; KOTHA SUBBARAMAIAH, Ph.D.; ANDREW J. DANNENBERG, M.D.

ABSTRACT

Background. The objective of this prospective cross-sectional study was to determine if cyclo-oxygenase-2, or COX-2, is overexpressed in the inflamed gingival tissue of patients diagnosed as having moderate-to-severe chronic periodontitis, or CP.
Methods. The authors evaluated clinical measures, crevicular fluid and gingival biopsy specimens from patients with moderate or severe CP (n = 16) and from healthy volunteers (n = 8). Patients were diagnosed as having CP based on clinical attachment loss, or CAL, of at least 5 millimeters at two sites in each quadrant and on evidence of alveolar bone loss as assessed from standard periapical or bitewing radiographs. Healthy patients exhibited no sites with CAL of more than 2 mm and no evidence of alveolar bone loss. The authors used standard techniques to perform biochemical measures.
Results. Levels of interleukin 1 beta, or IL-1β, in crevicular fluid were more than doubled in the CP group ($P < .05$). The amounts of COX-2 mRNA and protein also were elevated in gingival tissues from subjects with CP compared with those from healthy subjects. To gain further mechanistic insights, the authors conducted in vitro studies. The results showed that lipopolysaccharide and tumor necrosis factor alpha, or TNF-α, induced COX-2 in macrophages, while IL-1β and TNF-α induced COX-2 in oral epithelial cells.
Conclusions. Taken together, these results suggest that levels of COX-2 in gingivae reflect clinical measures of periodontitis and gingival inflammation.
Clinical Implications. The discovery of increased levels of COX-2 in inflamed gingival tissue suggests that COX-2 represents a pharmacological target for the prevention or treatment of CP.

Chronic periodontitis, or CP, is an infectious disease of the supporting structures of the teeth, characterized by destruction of bone and connective tissue. The oral cavity is continually exposed to bacteria, their exotoxins and endotoxins and xenobiotics, as well as physical stress. This results in a complex microenvironment within the periodontium, consisting of immune surveillance responses, cellular damage and repair, and the production of cytokines and other inflammatory mediators on a constant basis.[1] A persistent gram-negative infection with periodontal pathogens can shift this environment to a state of chronic inflammation. While specific oral pathogens and their virulence factors are essential for disease initiation and progression, the host response to infection also contributes to tissue destruction.[2]

Prostaglandin E$_2$, or PGE$_2$, appears to be involved in mediating the tissue destruction that occurs in CP. Elevated levels of PGE$_2$ are detected in the gingivae and gingival crevicular fluid, or GCF, of patients with CP.[3] In addition to inducing vasodilatation and capillary permeability, PGE$_2$ stimulates osteoclast bone resorption.[4] Nonsteroidal anti-inflammatory drugs, or NSAIDs, suppress PGE$_2$ in GCF and reduce periodontitis-induced bone loss in both animals and humans.[5-8] It is reasonable to postulate, therefore, that elucidating the molecular mechanisms that account for overproduction of PGE$_2$ in CP could lead to new therapeutic strategies.

The study results confirm the importance of measuring levels of COX-2 as a useful biomarker of gingival inflammation.

Two isoforms of cyclo-oxygenase, or COX, catalyze the synthesis of PGs from arachidonic acid. COX-1 is a housekeeping gene that is expressed constitutively in most tissues.[9] COX-2 is an immediate, early-response gene that is highly inducible by mitogenic and

inflammatory stimuli.[10-12] Elevated levels of COX-2 (enzyme) are detected in a variety of inflammatory conditions, including arthritis and inflammatory bowel disease.[13,14] Researchers have used immunohistochemistry to demonstrate increased levels of COX-2 protein in inflamed gingiva.[15,16] To further evaluate the potential significance of COX-2 in CP, we performed a prospective, cross-sectional study that compared clinical measures of CP severity with histologic and biochemical markers of gingival inflammation in patients with CP and in healthy volunteers.

SUBJECTS, MATERIALS AND METHODS

Clinical examination. Of the 24 systemically healthy adults enrolled in this study, 16 had moderate-to-severe CP[17] and eight volunteers did not have periodontitis. A single examiner (S.E.) performed all clinical measurements. The diagnosis of moderate or severe periodontitis required clinical attachment loss, or CAL, of at least 5 mm at two sites in each quadrant, with radiographic evidence of moderate or severe alveolar bone loss. We determined bone loss by visually interpreting standard dental radiographs. We used a full-mouth series of radiographs to evaluate all cases of periodontitis.

For healthy subjects, we used bite-wing radiographs. Bite-wing radiographs with distances of less than 2 mm from the alveolar crest to the cementoenamel junction, or CEJ, were considered to be evidence of no bone loss. We considered patients to be healthy if they had no sites with CAL of greater than 2 mm, and exhibited no evidence of alveolar bone loss. In addition, we required all patients with CP to have at least one site that exhibited clinical evidence of gingival inflammation for sampling. A gingival index, or GI, was used to assess inflamed sites, and scoring was done on a scale from 0 (absence of inflammation) to 3 (severe inflammation), according to established criteria.[18]

The examiner measured probing depth, or PD, with a manual probe. PD was defined as the distance in millimeters from the coronal-most margin of the free gingiva to the most apical penetration of the probe. We defined CAL as the distance from the CEJ to the most apical penetration of the probe.

None of the patients in our study had a history of recent periodontal treatment or long-term use of NSAIDs that, in the clinical judgment of the examiner, could have affected the outcome of the

study. We recorded subjects' smoking status. Exclusion criteria included the following: conditions that can affect infectious or inflammatory parameters such as rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, active tuberculosis, chronic osteomyelitis, HIV infection and nephrotic syndrome; active bacterial infectious diseases such as pneumonia or pyelonephritis within the previous two weeks; use of an antibiotic or selective COX-2 inhibitor within the previous six months; current use of heparin or coumadin; pregnancy; diabetes mellitus; and any condition necessitating antibiotic premedication for dental appointments.

The institutional review boards of Columbia University, New York, and the Weill Medical College of Cornell University, New York, approved the study, and all subjects gave their informed consent.

GCF analysis. The examiner collected a GCF specimen from the mesiobuccal and mesiopalatal sites adjacent to the biopsy site, as previously described.[19] Teeth were isolated, supragingival plaque was gently removed and samples of GCF were collected on precut methylcellulose filter paper strips for 30 seconds. We snap froze the strips, stored them at -70 C for three months and then analyzed them. The GCF samples were then eluted and assayed in duplicate for interleukin 1 beta, or IL-1β, with the use of a commercially available enzyme-linked immunosorbant assay (Immunotech, Marseille, France). We report the results as the total amount of IL-1ß in picograms per 30-second sample.[20]

Gingival biopsy. The examiner collected biopsy specimens from sites with the maximum GI score for each patient. For example, if the maximum GI score for a patient was 0, then the biopsy specimen was from a 0 site; if the maximum GI score was 1, then a biopsy specimen was taken from a 1 site. All biopsy specimens from patients with periodontitis had a GI score of 2 or 3; all specimens from healthy control subjects had a GI score of 0 or 1.

Once the biopsy site was determined, the examiner administered local anesthetic and removed interdental tissue, as previously described.[20] Two 3- to 4-mm pieces of tissue were obtained and fixed in 10 percent neutral-buffered formalin for histopathologic examination or snap frozen and stored at -70 C until analyzed. We used biopsy specimens from 18 subjects for quantitative reverse transcriptase–polymerase chain

TABLE

CLINICAL MEASURES, DEMOGRAPHICS AND LEVELS OF IL–1β* IN GCF†.			
VARIABLE	**HEALTHY CONTROL SUBJECTS (n = 8)**	**PATIENTS WITH CHRONIC PERIODONTITIS (n = 16)**	**_P_ VALUE‡**
Mean ± SEM§ Age (years)	28.8 ± 0.6	44.9 ± 3.4	.0036
Mean ± SEM Probing Depth at Biopsy Site (millimeters)	2.13 ± 0.12	5.8 ± 0.57	< .05
Mean ± SEM Gingival Index at Biopsy Site	0.75 ± 0.16	2.29 ± 0.11	< .001
Female Subjects (percentage)	37	43	–
Mean ± SEM IL-1β in GCF (picograms)¶	47.6 ± 17.2	120.6 ± 20.3	< .05

* IL-1β: Interleukin-1 beta.
† GCF: Gingival crevicular fluid.
‡ Comparison between groups (Student _t_ test).
§ SEM: Standard error of the mean.
¶ Total amount in picograms per 30-second sample.

reaction, or RT-PCR. Tissue from six (38 percent) of the 16 subjects with CP was used exclusively for immunoblot analysis. Several of the biopsy specimens were large enough for us to perform both COX-2 mRNA and immunoblot analyses.

Reagents and cell lines. Tumor necrosis factor alpha, or TNF-α, IL-1β and endotoxin (lipopolysaccharide, or LPS) (from _Escherichia coli_ 026:B6) were purchased from Sigma Chemical Co. (St. Louis). We obtained the cell culture media from Life Technologies (Grand Island, N.Y.). The murine macrophagelike cell line RAW 264.7 and 1483 oral squamous carcinoma cell lines were maintained as described in previous studies.[21,22]

Western blotting. We conducted immunoblot analysis for COX-2 as described in previous studies[23] using COX-2 polyclonal antiserum PG-27 (Oxford Biomedical Research, Oxford, Mich.) combined with enhanced chemiluminescent detection (ECL, Amersham, Piscataway, N.J.).

Quantitative PCR for COX-2 in gingival tissue. We isolated total RNA from biopsy tissue using RNeasy Mini Kits (Qiagen, Santa Clarita, Calif.). One microgram of total RNA was reverse transcribed with the use of the GeneAmp RNA PCR kit (Applied Biosystems, Foster City, Calif.) according to the manufacturer's protocol. We carried out quantitative RT-PCR as previously described,[23] using a competitor template as an

internal reference and COX-2 specific primers (Sigma/Genosys, The Woodlands, Texas).

Statistical analysis. We used the Student _t_ test to make comparisons between groups. A difference between groups of $P < .05$ was considered significant.

RESULTS

Levels of IL-1β and COX-2. Compared with healthy control subjects, patients with CP had elevated mean probing depths and GIs (Table). Moreover, using histologic evaluation, we confirmed the presence of inflammation in tissue biopsy specimens from patients with CP. Consistent with these findings, patients with CP had IL-1β levels in GCF that were more than double those seen in healthy control subjects (Table).

Of the 16 patients with CP, 10 were nonsmokers, five were current smokers and one was a former smoker. Among the eight healthy control subjects, six were nonsmokers, one was a current smoker and one was a former smoker. IL-1ß values were not significantly different between smokers and nonsmokers (data not shown). We measured IL-1β rather than PGE_2 levels as an independent measure of inflammation, because levels of IL-1β and PGE_2 in GCF are known to be highly correlated ($r = .81$, $P < .001$) in patients with CP.[24]

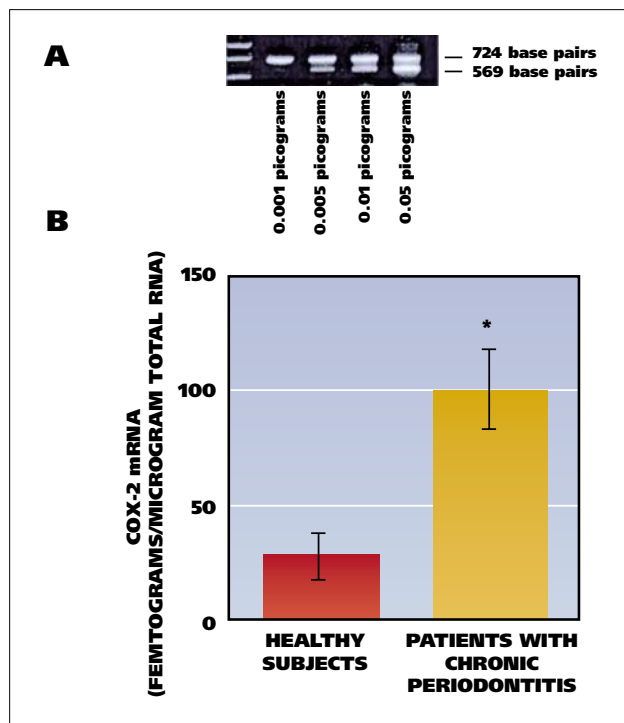We used quantitative RT-PCR to determine the amount of COX-2 mRNA in gingival samples.

Figure 1. Levels of cyclo-oxygenase-2, or COX-2, mRNA are increased in gingival tissue from patients with chronic periodontitis, or CP. A: A representative quantitative reverse transcriptase–polymerase chain reaction, or RT-PCR, in a case of CP. Total RNA (0.5 micrograms) and known concentrations (0.001-0.05 pg) of hCOX-2 mimic internal control are competing for a fixed amount of hCOX-2 primer in each reaction, giving rise to relative proportions of 569-bp internal control product and 724-bp target COX-2 cDNA product. B: Quantitative RT-PCR was used to determine the amounts of COX-2 mRNA in eight samples of gingivae from healthy subjects (mean ± standard error of the mean, or SEM, 28.0 ± 9.4 fg/μg of total RNA) and in 10 samples of gingivae from patients with CP (mean ± SEM, 99.3 ± 17.2 fg/μg of total RNA). The asterisk indicates $P < .01$.

This method relies on the coamplification in the same tube of known amounts of competitor DNA as an internal control, along with COX-2 cDNA obtained after reverse transcription from total-tissue RNA. The competitor and target use the same PCR primers, but yield amplicons with a different size (Figure 1A), allowing their separation on a gel at the end of the reaction. COX-2 mRNA was readily detectable in all tissue samples. Levels were increased by about 250 percent in the gingivae of patients with CP (Figure 1B).

Immunoblot analysis was performed on gingivae from seven subjects with CP and three healthy subjects. All of the biopsy specimens from patients with CP exhibited COX-2 protein. Moreover, patients with CP had higher levels of COX-2 protein than did those with healthy gingivae. Figure 2 shows a representative immunoblot.

Effects of LPS, TNF-α and IL-1β on the expression of COX-2 in macrophage and epithelial-derived cell lines. To further understand the mechanisms controlling the expression of COX-2 in CP, we compared the effects of *E. coli* LPS, IL-1β and TNF-α on levels of COX-2 in cultured cells. The effects of LPS, IL-1β and TNF-α were evaluated because of their recognized role in CP. LPS was a potent inducer of COX-2 in the RAW 264.7 macrophagelike cell line, but had little effect on amounts of COX-2 in the epithelial cell line (Figure 3). By contrast, IL-1β induced COX-2 effectively in epithelial cells but not in macrophages. COX-2 was induced by TNF-α in both cell lines.

DISCUSSION

The results of this cross-sectional study clearly demonstrate that the inflamed gingivae of patients with clinically defined CP exhibit increased levels of COX-2 mRNA and protein compared with the gingivae of healthy volunteers. These findings are consistent with previous reports, in which elevated amounts of COX-2 protein were detected immunohistochemically in inflamed gingivae.[15,16]

It is important to note that previous studies did not report clinical and biochemical measurements of periodontal disease severity. Thus, this report is the first to associate professionally accepted clinical measurements of disease severity with elevated amounts of COX-2 mRNA and protein in affected gingival tissue from patients with CP. We did not measure bacterial load or characterize the oral pathogens present. It is plausible that different species of periodontal pathogens will stimulate COX-2 expression to varying degrees.

A potential limitation of our study is that the patients with CP were somewhat older than the healthy volunteers (Table). Several recent studies have shown a relationship between aging and the expression of COX-2.[25-27] It is intriguing to speculate, therefore, that proinflammatory stimuli could lead to greater induction of COX-2 in older subjects. Future studies will need to control for age to determine the relative importance of this factor as a determinant of COX-2 expression in CP.

There is ample evidence that COX-2 can be a rate-limiting enzyme in the PG biosynthetic pathway. As discussed above, PGE_2 is considered to be an important mediator of the bone and tissue destruction observed in active periodontitis.[2,3] A number of nonselective COX

inhibitors have shown promise clinically for the treatment of CP.[6-8,28] However, Kelm and colleagues[29] believed that the risk of gastrointestinal side effects from long-term systemic administration of NSAIDs outweighed the benefit of using these agents to treat CP. Topical NSAID formulations were developed in an effort to avoid the gastrointestinal side effects associated with systemic use of nonselective NSAIDs.[29]

Three separate randomized, double-blind, placebo-controlled phase II clinical studies evaluated the safety and efficacy of an oral rinse formulation of the potent COX-1/COX-2 inhibitor, ketorolac tromethamine.[30] A surprising oral mucosal finding was observed in patients with CP and gingivitis in these studies. Specifically, after 12 months of treatment, the researchers observed a statistically significant dose-response relationship ($P < .05$) in the incidence of reported mouth ulcerations compared with the incidence when placebo rinse was used.[30] Except for these infrequent oral soft-tissue findings, ketorolac tromethamine rinse was generally well-tolerated.

Selective COX-2 inhibitors are widely used to treat arthritis and acute postsurgical dental pain.[31,32] Fewer serious gastrointestinal side effects were found with COX-2 inhibitors than with traditional NSAIDs.[33,34] Likewise, it is reasonable to postulate that topically applied selective COX-2 inhibitors might not be associated with oral mucosal ulcerations. Based on the results of our current study, it will be important to determine whether selective COX-2 inhibitors administered systemically or topically are useful in the treatment of patients with CP.

Another interesting question raised by our findings is with regard to the factors regulating the expression of COX-2 in healthy versus inflamed gingivae. The mean concentration of COX-2 mRNA in healthy gingival tissue (28 femtograms/microgram of total RNA) was approximately 10-fold higher than the level (2.4 fg/µg of total RNA) we previously observed in healthy nongingival oral mucosa.[23] It is possible that levels of COX-2 are higher in healthy gingivae than in other healthy oral tissues because of bacterial colonization or mechanical stress.[35] It would be worthwhile to determine whether other genes involved in CP, such as matrix metalloproteinases, also are expressed at higher levels in healthy gingivae than in other oral tissues.

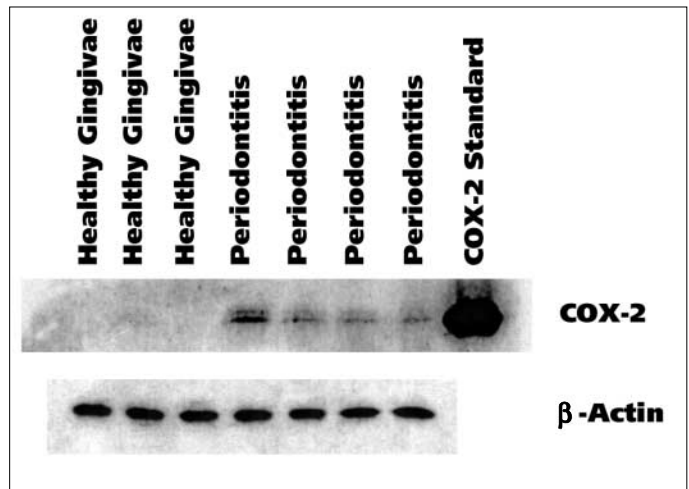The continued expression of COX-2 (and possibly other genes) over time or its increase with a

Figure 2. Increased levels of cyclo-oxygenase-2, or COX-2, protein in gingival tissue from patients with chronic periodontitis, or CP. The authors performed immunoblotting on gingivae from three healthy volunteers and from four patients with CP. Tissue lysate protein (100 micrograms/lane) was loaded onto a 10 percent sodium dodecyl sulfate–polyacrylamide gel and electrophoresed. The immunoblot was probed sequentially with antibodies to COX-2 and β-actin. Ovine COX-2 was used as a standard.
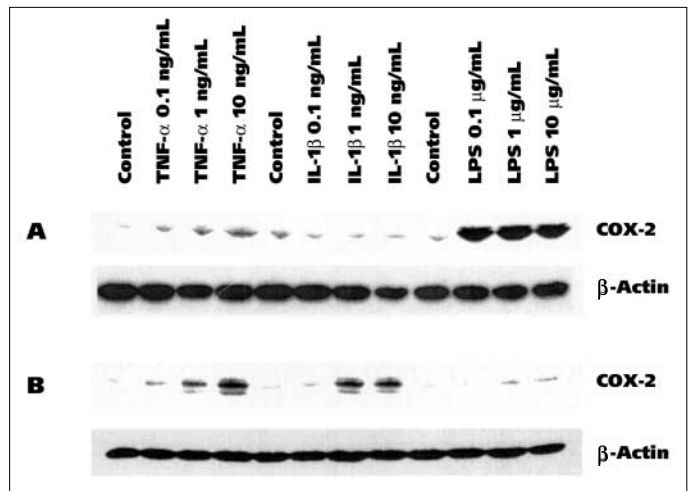

Figure 3. Effects of tumor necrosis factor alpha, or TNF-α, interleukin 1 beta, or IL-1β, and lipopolysaccharide, or LPS, on the expression of cyclo-oxygenase-2, or COX-2. Lysate protein was from RAW 264.7 (panel A) or 1483 (panel B) cells treated with vehicle (control), TNF-α, IL-1β or LPS for six hours. Cellular lysate protein (30 micrograms/lane) was loaded onto a 10 percent sodium dodecyl sulfate–polyacrylamide gel, electrophoresed and transferred onto nitrocellulose. Immunoblots were probed with antibodies specific for COX-2 and β-actin. ng/mL: Nanograms per milliliter.

patient's age could contribute to a subclinical inflammatory state leading to progressive attachment loss. This would be consistent with the known relationship between attachment loss and age, suggesting that topical anti-inflammatory
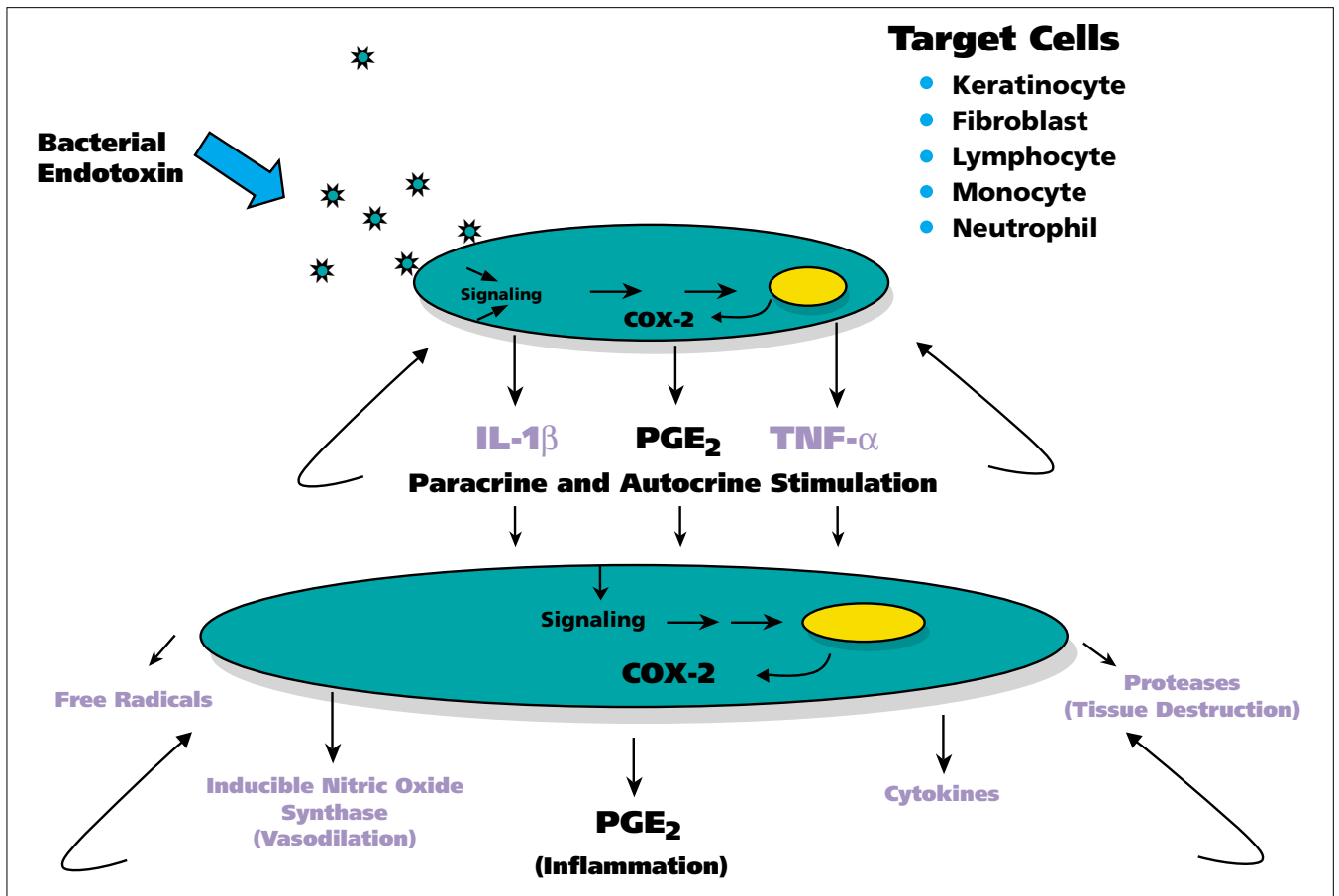
Figure 4. Schematic depicting the stimulation of cyclo-oxygenase-2, or COX-2, expression in the periodontium by paracrine and autocrine factors produced as part of the inflammatory cascade initiated by periodontal pathogens. IL-1β: Interleukin 1 beta. PGE$_2$: Prostaglandin E$_2$. TNF-α: Tumor necrosis factor alpha.

agents could play a valuable role in routine oral hygiene.

We observed a further increase in levels of COX-2 in inflamed gingivae. Bacterial toxins are key drivers of the inflammatory signal transduction cascade observed in gingivitis and periodontitis. The periodontal pathogen *Porphyromonas gingivalis* has been demonstrated to stimulate the expression of COX-2 and production of PGE$_2$ in experimental models of inflammation.[36] The potential importance of bacterial endotoxin is suggested by our in vitro findings. It is important to note that LPS induced COX-2 in macrophages.

Endotoxin also is known to stimulate the production of IL-1β and TNF-α by macrophages. It is significant, therefore, that IL-1β was a potent inducer of COX-2 in epithelial cells but not in macrophages. By contrast, COX-2 was induced by TNF-α in both macrophages and oral epithelial cell lines. Taken together, these results suggest

that endotoxin initiates an inflammatory cascade whereby COX-2 is induced by both autocrine and paracrine mechanisms (Figure 4). Experiments using gingival fibroblasts suggest a role for specific tyrosine kinases in the regulation of COX-2 by cytokines in this cell type.[37] Additional studies are needed to compare the effects of LPS produced by different periodontal pathogens and to define the signal transduction pathway by which COX-2 is induced in CP.

CONCLUSION

Both COX-2 mRNA and protein levels are significantly elevated in inflamed gingival tissue from patients with CP compared with levels in uninflamed tissue from healthy subjects. We defined the tissue from patients with CP as inflamed using clinical, biochemical and histologic criteria. These results confirm the importance of measuring levels of COX-2 as a useful biomarker of gingival inflammation. Our study also raises the

possibility that COX-2 represents a valid pharma-cologic target for the treatment, prevention or both of periodontitis. ■

Dr. Zhang is a postdoctoral fellow, Department of Genetic Medicine, Weill Medical College of Cornell University, New York City.

Dr. Engebretson is an assistant professor of dentistry, Division of Periodontics, Columbia University School of Dental and Oral Surgery, New York City.

Dr. Morton is a periodontist in private practice in New York City.

Dr. Cavanaugh is a senior research scientist, Oral Health Care Technology Division, The Procter and Gamble Co., Cincinnati.

Dr. Subbaramaiah is an associate professor, Department of Medicine, Weill Medical College of Cornell University, and Strang Cancer Prevention Center, New York City.

Dr. Dannenberg is the Henry R. Erle, M.D.—Roberts Family Professor of Medicine, Weill Medical College of Cornell University, 1300 York Ave., Room F-206, New York, N.Y. 10021, e-mail "ajdannen@med.cornell.edu". Address reprint requests to Dr. Dannenberg.

1. Bartold PM, Walsh LJ, Narayanan AS. Molecular and cell biology of the gingiva. Periodontology 2000;24:28-55.
2. Offenbacher S, Heasman PA, Collins JG. Modulation of host PGE_2 secretion as a determinant of periodontal disease expression. J Periodontol 1993;64:432-44.
3. Offenbacher S, Odle BM, Van Dyke TE. The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. J Periodont Res 1986;21:101-12.
4. Rifkin BR, Baker RL, Coleman SJ. Effects of prostaglandin E_2 on macrophages and osteoclasts in cultured fetal long bones. Cell Tissue Res 1980;207:341-6.
5. Williams RC, Jeffcoat MK, Kaplan ML, Goldhaber P, Johnson HG, Wechter WJ. Flurbiprofen: a potent inhibitor of alveolar bone resorption in beagles. Science 1985;227:640-2.
6. Jeffcoat MK, Page R, Reddy M, et al. Use of digital radiography to demonstrate the potential of naproxen as an adjunct in the treatment of rapidly progressive periodontitis. J Periodont Res 1991;26:415-21.
7. Reddy MS, Palcanis KG, Barnett ML, Haigh S, Charles CH, Jeffcoat MK. Efficacy of meclofenamate sodium (MECLOMEN) in the treatment of rapidly progressive periodontitis. J Clin Periodontol 1993;20:635-40.
8. Jeffcoat MK, Reddy MS, Haigh S, et al. A comparison of topical ketorolac, systemic flurbiprofen, and placebo for the inhibition of bone loss in adult periodontitis. J Periodontol 1995;66:329-38.
9. Smith WL, Garavito RM, DeWitt DL. Prostaglandin endoperoxide H synthases (cyclooxygenases)–1 and –2. J Biol Chem 1996;271:33157-160.
10. DuBois RN, Awad J, Morrow J, Roberts LJ, Bishop PR. Regulation of eicosanoid production and mitogenesis in rat intestinal epithelial cells by transforming growth factor-α and phorbol ester. J Clin Invest 1994;93:493-8.
11. Subbaramaiah K, Telang N, Ramonetti JT, et al. Transcription of cyclooxygenase-2 is enhanced in transformed mammary epithelial cells. Cancer Res 1996;56:4424-9.
12. Rhee SH, Hwang D. Murine TOLL-like receptor 4 confers lipopolysaccharide responsiveness as determined by activation of NFκB and expression of the inducible cyclooxygenase. J Biol Chem 2000;275:34035-40.
13. Crofford LJ, Wilder RL, Ristimaki AP, et al. Cyclooxygenase-1 and -2 expression in rheumatoid synovial tissues. J Clin Invest 1994;93:1095-101.
14. Singer II, Kawka DW, Schloemann S, Tessner T, Riehl T, Stenson WF. Cyclooxygenase-2 is induced in colonic epithelial cells in inflammatory bowel disease. Gastroenterology 1998;115:297-306.
15. Cavanaugh PF Jr., McDonald JS, Pavelic L, Limardi RJ, Gluckman JL, Pavelic ZP. Immunohistochemical localization of prostaglandin H synthase isoenzyme proteins in the gingival tissue of patients with periodontitis. Inflammopharmacology 1995;3:109-19.
16. Morton RS, Dongari-Bagtzoglou AI. Cyclooxygenase-2 is upregulated in inflamed gingival tissues. J Periodontol 2001;72:461-9.
17. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999;4:1-6.
18. Löe H, Silness J. Periodontal disease in pregnancy, I: prevalence and severity. Acta Odontol Scand 1963;21:533-51.
19. Engebretson SP, Grbic JT, Singer RE, Lamster IB. GCF IL-1β profiles in periodontal disease. J Clin Periodontol 2002;29:48-53.
20. Engebretson SP, Lamster IB, Herrera-Abreu M, et al. The influence of interleukin gene polymorphism on expression of interleukin-1β and tumor necrosis factor-α in periodontal tissue and gingival crevicular fluid. J Periodontol 1999;70:567-73.
21. Mestre JR, Subbaramaiah K, Sacks PG, et al. Retinoids suppress phorbol ester-mediated induction of cyclooxygenase-2. Cancer Res 1997;57:1081-5.
22. Fujita J, Mestre JR, Zeldis JB, Subbaramaiah K, Dannenberg AJ. Thalidomide and its analogues inhibit lipopolysaccharide-mediated induction of cyclooxygenase-2. Clin Cancer Res 2001;7:3349-55.
23. Chan G, Boyle JO, Yang EK, et al. Cyclooxygenase-2 expression is up-regulated in squamous cell carcinoma of the head and neck. Cancer Res 1999;59:991-4.
24. Cavanaugh PF Jr., Meredith MP, Buchanan W, Doyle MJ, Reddy MS, Jeffcoat MK. Coordinate production of PGE2 and IL-1β in the gingival crevicular fluid of adults with periodontitis: its relationship to alveolar bone loss and disruption by twice daily treatment with ketorolac tromethamine oral rinse. J Periodont Res 1998;33(2):75-82.
25. Ohzeki K, Yamaguchi M, Shimizu N, Abiko Y. Effect of cellular aging on the induction of cyclooxygenase-2 by mechanical stress in human periodontal ligament cells. Mech Ageing Dev 1999;108:151-63.
26. Kim HJ, Kim KW, Yu BP, Chung HY. The effect of age on cyclooxygenase-2 gene expression: NF-κB activation and IκBα degradation. Free Radic Biol Med 2000;28:683-92.
27. Shimizu N, Yamaguchi M, Uesu K, Goseki T, Abiko Y. Stimulation of prostaglandin E_2 and interleukin-1beta production from old rat periodontal ligament cells subjected to mechanical stress. J Gerontol A Biol Sci Med Sci 2000;55:B489-95.
28. Williams RC, Jeffcoat MK, Howell TH, et al. Altering the progression of human alveolar bone loss with the non-steroidal anti-inflammatory drug flurbiprofen. J Periodontol 1989;60:485-90.
29. Kelm GR, Buchanan W, Meredith M, et al. Evaluation of ketorolac concentrations in plasma and gingival crevicular fluid following topical treatment with oral rinses and dentifrices. J Pharm Sci 1996;85:842-7.
30. Cavanaugh PF Jr., Meredith MP, Gerlach RW. The use of ketorolac tromethamine oral rinse for the treatment of periodontitis in adults. Inflammopharmacology 1998;5:204-5.
31. Vioxx prescribing information. Available at: "www.vioxx.com". Accessed May 19, 2003.
32. Celebrex prescribing information. Available at: "www.celebrex.com". Accessed May 19, 2003.
33. Langman MJ, Jensen DM, Watson DJ, et al. Adverse upper gastrointestinal effects of rofecoxib compared with NSAIDs. JAMA 1999;282:1929-33.
34. Fitzgerald GA, Patrono C. The COXIBS, selective inhibitors of cyclooxygenase-2. N Engl J Med 2001;345:433-42.
35. Shimizu N, Ozawa Y, Yamaguchi M, Goseki T, Ohzeki K, Abiko Y. Induction of COX-2 expression by mechanical tension force in human periodontal ligament cells. J Periodontol 1998;69:670-7.
36. Pouliot M, Clish CB, Petasis NA, Van Dyke TE, Serhan CN. Lipoxin A_4 analogues inhibit leukocyte recruitment to *Porphyromonas gingivalis*: a role for cyclooxygenase-2 and lipoxins in periodontal disease. Biochemistry 2000;39:4761-8.
37. Yucel-Lindberg T, Ahola H, Carlstedt-Duke J, Modéer T. Involvement of tyrosine kinases on cyclooxygenase expression and prostaglandin E_2 production in human gingival fibroblasts stimulated with interleukin-1β and epidermal growth factor. Biochem Biophys Res Comm 1999;257:528-32.