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Prostaglandin E_2 Inhibits the Proliferation of Human Gingival Fibroblasts Via the EP_2 Receptor and Epac

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ABSTRACT

Elevated levels of prostaglandins such as PGE_2 in inflamed gingiva play a significant role in the tissue destruction caused by periodontitis, partly by targeting local fibroblasts. Only very few studies have shown that PGE_2 inhibits the proliferation of a gingival fibroblast (GF) cell line, and we expanded this research by using primary human GFs (hGFs) and looking into the mechanisms of the PGE_2 effect. GFs derived from healthy human gingiva were treated with PGE_2 and proliferation was assessed by measuring cell number and DNA synthesis and potential signaling pathways were investigated using selective activators or inhibitors. PGE_2 inhibited the proliferation of hGFs dose-dependently. The effect was mimicked by forskolin (adenylate cyclase stimulator) and augmented by IBMX (a cAMP-breakdown inhibitor), pointing to involvement of cAMP. Indeed, PGE_2 and forskolin induced cAMP generation in these cells. Using selective EP receptor agonists we found that the anti-proliferative effect of PGE_2 is mediated via the EP_2 receptor (which is coupled to adenylate cyclase activation). We also found that the effect of PGE_2 involved activation of Epac (exchange protein directly activated by cAMP), an intracellular cAMP sensor, and not PKA. While serum increased the amount of phospho-ERK in hGFs by ~300%, PGE_2 decreased it by ~50%. Finally, the PGE_2 effect does not require endogenous production of prostaglandins since it was not abrogated by two COX-inhibitors. In conclusion, in human gingival fibroblasts PGE_2 activates the EP_2–cAMP–Epac pathway, reducing ERK phosphorylation and inhibiting proliferation. This effect could hamper periodontal healing and provide further insights into the pathogenesis of inflammatory periodontal disease. J. Cell. Biochem. 108: 207–215, 2009.

KEY WORDS: PROSTAGLANDINS; GINGIVAL FIBROBLASTS; EP_2 RECEPTOR; EPAC

Periodontal disease is caused by the interactions between a chronic bacterial insult and host responses [Page et al., 1997; Madianos et al., 2005]. The inflammatory process, which results from this interaction, is largely responsible for the destruction of the periodontium (gingiva and alveolar bone). The major factors driving the inflammatory/destructive process are virulence factors produced by the bacteria (e.g., LPS), as well as locally produced cytokines (e.g., TNFα and IL-1) and prostanoids [Madianos et al., 2005]. TNFα and IL-1 can induce gingival tissue damage by stimulating the production of other inflammatory mediators (e.g., prostaglandins) and induction of matrix metalloproteinase (MMP) production and activation (leading to extracellular matrix destruction) [Graves and Cochran, 2003]. Prostaglandins, the levels of which increase significantly in inflamed gingiva [Dewhirst et al., 1983; Offenbacher et al., 1989], are potent inducers of osteoclast generation and consequent alveolar bone resorption and as such have long been implicated in the destruction of the periodontium [Williams et al., 1987; Howell and Williams, 1993] in inflamed loci.

In addition, significant cellular death is common in inflamed gingival connective tissue [Zappa et al., 1992; Nemeth et al., 1993; Tonetti et al., 1998], and this can be caused by several components of the periodontal inflammatory process via their effect on gingival fibroblasts, that is, induction of their apoptosis [Alikhani et al., 2004], or inhibition of their proliferation [ElAttar and Lin, 1993; Arai et al., 1995], thus also hampering tissue repair. Among the prostanoids found in inflamed gingiva, prostaglandin E_2 (PGE_2) has documented anti-proliferative effects in tendon [Thampatty et al., 2006], lung [Huang et al., 2007], embryonic [Hsi and Eling, 1998], hepatic [Koide et al., 2004], and gingival [Arai et al., 1995, 1998] fibroblasts as well as other cell types.

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There is very limited information on the mechanisms whereby PGE_2 inhibits the proliferation of fibroblasts in general and gingival fibroblasts in particular. PGE_2 acts on a variety of cells via cell-surface G-protein-coupled receptors divided into 4 subtypes, EP_{1-4}, which differ in their signal transduction pathways. Of these 4 receptors, EP_4 and EP_2 activate adenylate cyclase and increase intracellular levels of cAMP, EP_1 activates phospholipase C (PLC) and EP_3 either lowers cAMP levels or activates PLC, depending on the alternatively spliced isoform [Abramovitz et al., 1995; Boie et al., 1997]. While several reports show that PGE_2 inhibition of proliferation is mediated via increased cAMP production, implicating EP_2 or EP_4 in the effect, no data are available on EP involvement in gingival fibroblasts. Furthermore, there are conflicting reports as to the pathways downstream to cAMP that lead to suppression of proliferation, in particular the involvement of cAMP-dependent protein kinase (PKA).

Therefore this study explored the anti-proliferative effect of PGE_2 on primary human gingival fibroblasts in terms of the EP receptor involved and some of the post-receptor signal transduction pathways affected by PGE_2, in particular cAMP and PKA.

MATERIALS AND METHODS

MATERIALS

All reagents for tissue culture were from Biological Industries (Beit Haemek, Israel). Tissue culture dishes were from Nunc (Rosekilde, Denmark), Crystal violet from Edward Gurr (London, UK), Trichloracetic acid (TCA) and IBMX (a phosphodiesterase inhibitor which prevents cAMP breakdown), were from Sigma (St. Louis, MO). (^{3}H)Thymidine was from Perkin Elmer (Boston) while Forskolin, NS-398 (COX-2 inhibitor), Indomethacin (general COX inhibitor) and H-89 (PKA inhibitor) were from Alexis Biochemicals (Lausen, Switzerland). PGE_2 was from Biomol (Plymouth Meeting, PA) and was dissolved in 100% ethanol at 10^{-2} M and further diluted in culture medium, so that the final ethanol concentration was never toxic to the cells (less than 0.1%). ONO-DI-004 (EP_1 agonist), ONO-AE1-259-01 (EP_2 agonist), ONO-AE-248 (EP_3 agonist), ONO-AE1-329 (EP_4 agonist), and ONO-AE3-208 (EP_4 antagonist) [Suzawa et al., 2000; Sugimoto and Narumiya, 2007] were a generous gift from ONO Pharmaceutical Corporation and were dissolved in DMSO or in H_2O.

8-(4-Chlorophenylthio)-2′-O-methyladenosine-3′, 5′-cyclic monophosphate (8-pCPT-2′-O-Me-cAMP, a selective Epac activator [Enserink et al., 2002]), Adenosine-3′, 5′-cyclic monophosphorothioate, Rp-isomer (Rp-cAMPS, PKA inhibitor [Gjertsen et al., 1995]), and N^6-Benzoyladenosine-3′, 5′-cyclic monophosphate (6-Bnz-cAMP, a selective activator of PKA but not Epac [Bos, 2006]) were from BIOLOG Life Science Institute (Bremen, Germany). In preliminary experiments we determined that the concentrations of all the solvents used (ethanol, DMSO and H_2O) had no effect on cell number or DNA synthesis.

Antibodies to phospho-ERK and ERK (both recognizing ERK-1 and ERK-2) were from Sigma. Peroxidase-conjugated anti-mouse or anti-rabbit IgG, respectively, was from Jackson (West Grove).

CELL ISOLATION AND CULTURE

The experiments were approved by the Helsinki committee of the Tel-Aviv University and informed consent was obtained from all patients. Gingival tissue was removed during periodontal or implant procedures, the epithelium was removed and connective tissue fragments were cut into small pieces and placed in culture medium (α-MEM supplemented with 10% fetal calf serum (FCS), 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 12.5 U/ml nystatin, 0.11 mg/ml sodium pyruvate, and non-essential amino acids) at 37°C in a humidified atmosphere of 5% CO_2 and 95% air to allow for cell outgrowth. The medium was replaced every 3 days until confluence was reached. Cells at the second or third passage, having a typical fibroblastic morphology, were used.

CELL NUMBER

Cell number was determined colorimetrically using crystal violet [Saati et al., 1997]. Cells were plated at 50,000 cells/well in 24-well plates in triplicates, and allowed to attach and spread for 24 h in a medium containing 10% FCS. Cells were then starved for 24 h in a serum-free medium and then challenged with different medium combinations (5% FCS as proliferation stimulant + various additives) and cell number was determined 48 h later. Cells were washed with PBS, fixed in 70% ethanol and stained with 1% crystal violet. Unincorporated stain was removed by washing, cells were air-dried and the dye extracted with 70% ethanol and its absorbance (550 nm) was measured in a Microplate Reader (Spectra max 190, Molecular Devices, USA). Calibration curves showed that cell number is linearly correlated to optical density of the dye.

THYMIDINE INCORPORATION

Thymidine incorporation was assayed as described previously [Zeldich et al., 2007a]. Twenty hours after challenging the cells, (^{3}H) thymidine was added at a final concentration of 1 μCi/ml for 4 h, and cells were washed three times with PBS. DNA was precipitated with 5% TCA for 45 min on ice, solubilized with 0.5 N NaOH for 90 min at room temperature and the radioactivity in the cell lysate was determined in a Beckman® LS-6000SC Liquid Scintillation Counter.

CAMP MEASUREMENTS

Intracellular cAMP measurements were performed with the cAMP Biotrak EIA kit from Amersham (Little Chalfont, UK). Cells (50,000 per well) were incubated with 5% FCS ± IBMX for 15 min, after which PGE_2 was added for 15 min, followed by lysis and measurements according to the manufacturer's instructions.

WESTERN BLOT ANALYSIS

Cells (500,000 per 60-mm culture dish) were treated with 5% FCS ± PGE_2 for 5–22 h and then washed with ice-cold PBS, lyzed with SDS-sample buffer and boiled for 15 min. Samples were subjected to SDS–PAGE under reducing conditions using 10% polyacrylamide gels (20 μg protein per lane) on a TransBlot SD device (Bio-Rad, Hercules, CA). Proteins were transferred to nitrocellulose membranes and probed for 2 h at room temperature with specific primary antibodies. No primary antibody was used for negative control. Bound antibodies were visualized using a

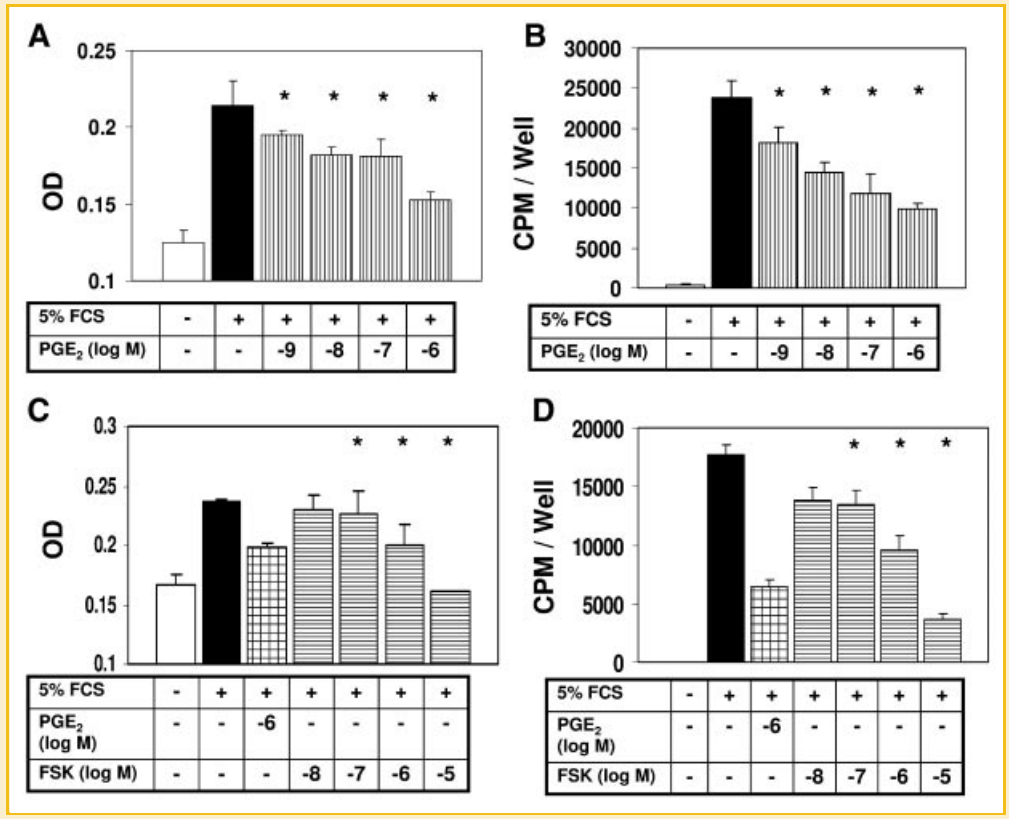

Fig. 1. PGE_2 (A,B) and forskolin (FSK, C,D) inhibit serum-stimulated proliferation of hGFs. A,C: Cell number (measured colorimetrically by crystal violet staining and presented as optical density (OD)) 48 h after stimulation with fetal calf serum (FCS) ± PGE_2; (B,D) DNA synthesis (measured as thymidine incorporation) 24 h after stimulation with FCS ± PGE_2. $^*P < 0.05$ versus control (FCS without any additives). In all subsequent figures, PGE_2 at 1 μM is used as positive control. N = 3–4 wells per condition.

horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence reagents (Pierce, Rockford) and BioMax light film (Kodak, USA).

STATISTICAL ANALYSIS

All assays were performed in triplicates/quadruplicates for each condition and each experiment was repeated at least twice. The results are presented as mean and standard deviation (SD). Statistical analysis was performed by ANOVA followed by post-hoc multiple comparisons using the Tukey tests.

RESULTS

Switching hGFs to a medium containing 5% FCS increased cell number by ~75% within 48 h, an effect which was gradually inhibited by increasing concentrations of PGE_2 within the range of 1 nM to 1 μM (Fig. 1A). Similarly, thymidine incorporation (measured at 24 h) was stimulated ~50-fold by 5% FCS and this was dose-dependently inhibited by PGE_2 (Fig. 1B). In all subsequent experiments, PGE_2 at 1 μM was used as positive control.

Forskolin (10 nM to 10 μM) also inhibited the proliferation of hGFs (measured as cell number and thymidine incorporation) dose-dependently (Fig. 1C,D). In addition, IBMX (a phosphodiesterase inhibitor) augmented dose-dependently the effect of each concentration of PGE_2 (0.001–0.1 μM) on proliferation of hGFs (Fig. 2A), suggesting that the effect of PGE_2 is mediated via increased cAMP levels. Indeed, both PGE_2 (at 0.1–1 μM) and forskolin (10 μM) dramatically increased in hGFs the intracellular levels of cAMP (Fig. 2B) while the addition of 5% FCS did not. Moreover, IBMX increased cAMP levels at every PGE_2 concentration tested (0.001–0.1 μM, Fig. 2C). These data indicate that PGE_2 inhibits the proliferation of human gingival fibroblasts via generation of cAMP.

In agreement with these findings, agonists of the EP_1 and EP_3 prostanoid receptors, which do not signal through elevated cAMP levels, had no significant effect on cell number or DNA synthesis of hGFs (Fig. 3A–D). An agonist of the EP_4 receptor, which signals through elevated cAMP, had no significant effect on cell number and a minor effect on DNA synthesis (Fig. 4A,B); however the EP_4 antagonist we employed did not alter the effect of PGE_2 on both parameters of proliferation (Fig. 4C,D), indicating that EP_4 is not the major receptor mediating the anti-proliferative effect of PGE_2. In contrast, an agonist of the EP_2 receptor, which also signals via elevated cAMP, reduced cell number and thymidine incorporation down to the level obtained with PGE_2 (Fig. 5A,B). These data show that PGE_2 inhibits the proliferation of hGFs via the EP_2 prostanoid receptor, coupled to increased cAMP generation.

Both H89 and Rp-cAMPS (PKA inhibitors) failed to abrogate the PGE_2-induced inhibition of proliferation (Fig. 6A,B), and

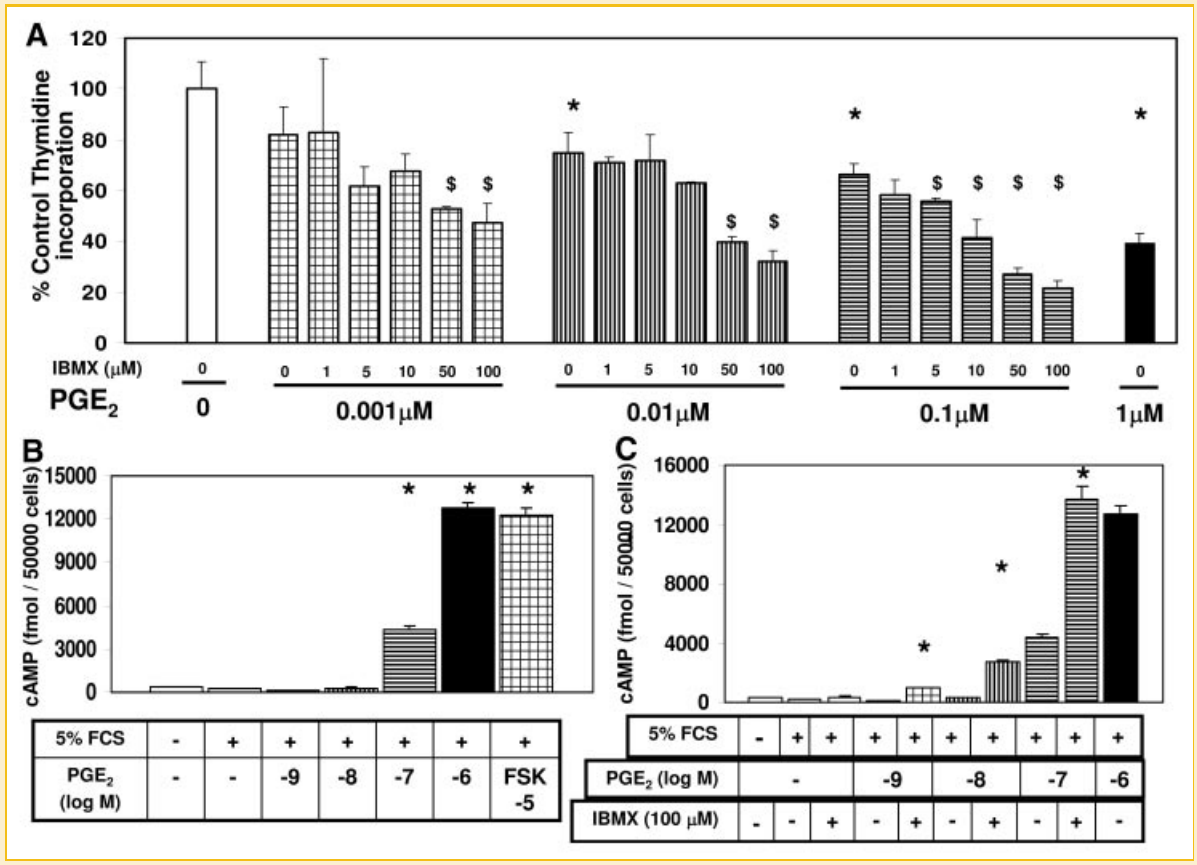

Fig. 2. A: IBMX (1–100 μM) augments the inhibitory effect of PGE_2 on DNA synthesis of hGFs (at 24 h poststimulation). $^{*}P < 0.05$ (PGE_2 without IBMX vs. control ($PGE_2 = 0$)); $^{\$}P < 0.05$ (IBMX concentrations vs. the respective IBMX = 0 of the same PGE_2 concentration). IBMX alone had a negligible (<10%) effect on thymidine incorporation. N = 3–4 wells per condition. B: PGE_2 and forskolin (FSK) cause cAMP production in hGFs (measured 15 min poststimulation). $^{*}P < 0.05$ versus FCS alone; (C) IBMX (100 μM) increases cAMP levels (measured 15 min poststimulation) caused by each concentration of PGE_2. FCS, fetal calf serum. $^{*}P < 0.05$ (vs. no IBMX at the same PGE_2 concentration). N = 3–4 wells per condition.

6-Bnz-cAMP (a PKA activator) did not reduce GF proliferation (data not shown), indicating that the anti-proliferative effect of PGE_2 does not involve PKA activation as a downstream pathway secondary to cAMP elevation. In contrast, 8-pCPT-2′-O-Me-cAMP, a selective Epac activator, mimicked the effect of PGE_2 and dose-dependently inhibited hGF proliferation (Fig. 6C), suggesting that increased cAMP levels signal an anti-proliferative message via Epac, rather than through PKA in these cells. Serum treatment increased the amount of phospho-ERK in hGFs by ~300%, and PGE_2 decreased it by ~50% (Fig. 7) in parallel with their respective and contrasting effects on proliferation.

Finally, the anti-proliferative effect of PGE_2 on hGFs does not seem to require endogenous production of prostanoids in these cells, since 2 inhibitors of cyclooxigenase (COX) (indomethacin, a nonselective inhibitor of COX1 and COX2 and NS-398, a selective inhibitor of COX-2) failed to abrogate the effect of PGE_2 on hGF proliferation (Fig. 8A,B).

DISCUSSION

Our data clearly show that PGE_2 inhibits the serum-stimulated proliferation of human gingival fibroblasts. This finding is in line with other reports of anti-proliferative effects of PGE_2 on fibroblasts from a variety of sources and other cell types. In terms of gingival fibroblasts, these data corroborate and extend those of Arai et al. [1995, 1998], who used a GF cell line, to primary human GFs.

This is the first report to elucidate some of the mechanisms whereby PGE_2 inhibits GF proliferation. We believe that PGE_2 exerts its anti-mitogenic effect on hGFs through the following sequence of events: Binding of the EP_2 prostanoid receptor, followed by activation of adenylate cyclase and generation of intracellular cAMP, followed by activation of Epac and reduction of ERK phosphorylation.

Firstly, the effect of PGE_2 is clearly mediated via cAMP generation, since it was mimicked by forskolin, an adenylate cyclase activator, and augmented by IBMX, a phosphodiesterase inhibitor, which prevents the degradation of cAMP. These data are in agreement with those of Arai [1998], who used a GF-cell line, as well as other studies in which PGE_2 inhibits the proliferation of other fibroblasts [Hsi and Eling, 1998; Koide et al., 2004; Liu et al., 2004; Huang et al., 2007] via cAMP production. Out of the four E-type prostanoid receptors, two use cAMP as secondary messenger: EP_2 and EP_4. Previous studies showed that human GFs express both EP_2 and EP_4 [Noguchi et al., 2000, 2002]. Although we found a small

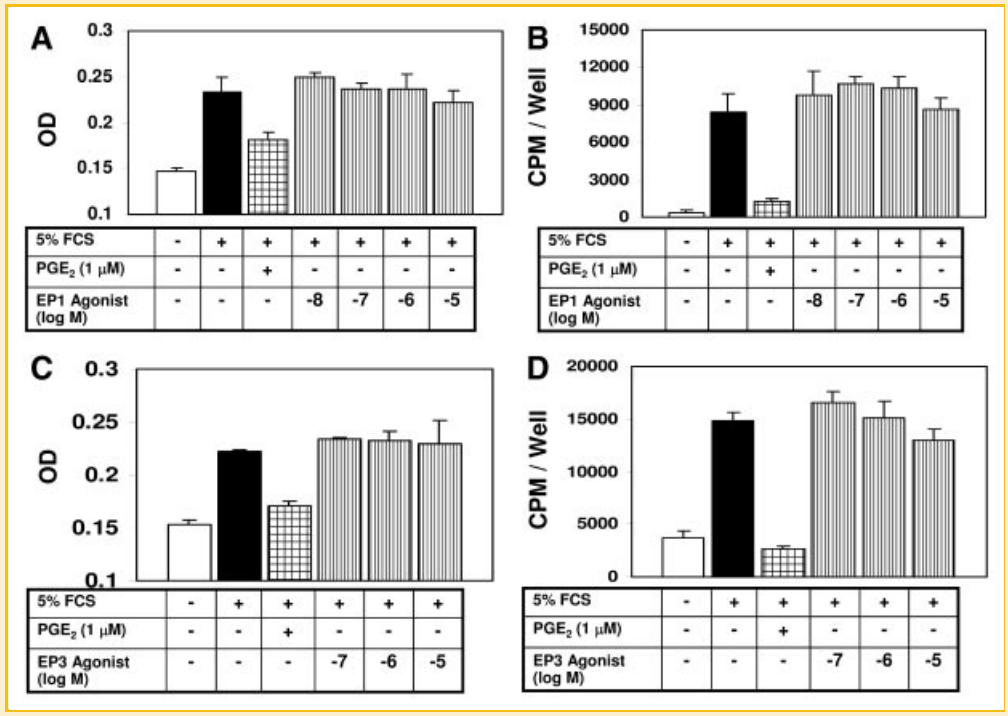

Fig. 3. Agonists of EP_1 (A,B) and EP_3 (C,D) do not affect fetal calf serum (FCS)-stimulated hGF proliferation, depicted as cell number at 48 h post stimulation (A,C) or thymidine incorporation at 24 h poststimulation (B,D). One micromolar PGE_2 is used as positive control. N = 3–4 wells per condition.

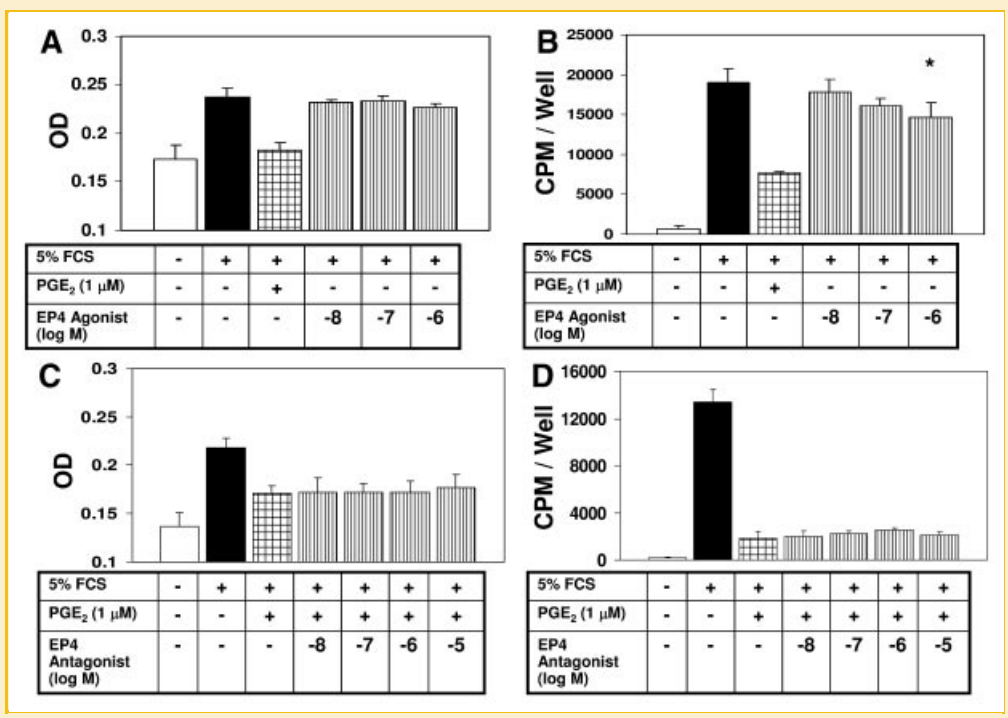

Fig. 4. An EP_4 agonist has no effect on fetal calf serum (FCS)-stimulated hGF cell number at 48 h (A) and a marginal effect on thymidine incorporation at 24 h (B). $^*P < 0.05$ versus control (FCS alone). One micromolar PGE_2 is used as positive control. C,D: An EP_4 antagonist does not prevent the anti-mitogenic effect of 1 μM of PGE_2 estimated by cell number at 48 h (C) and thymidine incorporation at 24 h (D). N = 3–4 wells per condition.

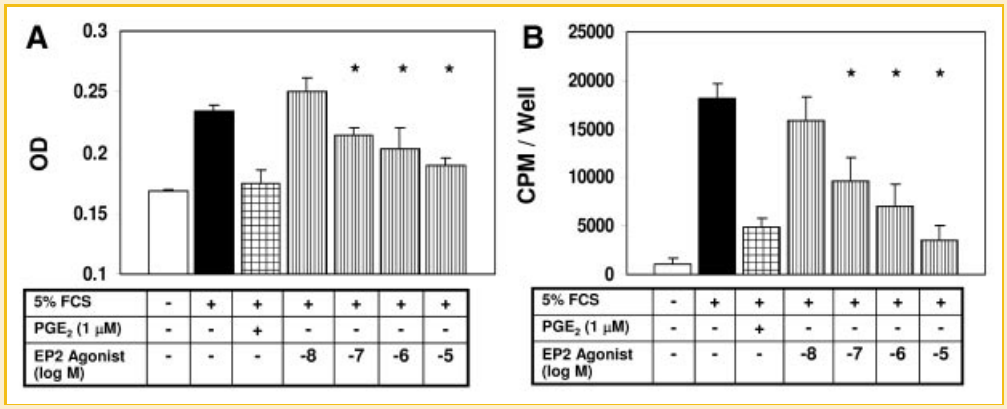

Fig. 5. The EP_2 agonist dose-dependently inhibits fetal calf serum (FCS)-stimulated hGF proliferation, depicted as cell number at 48 h (A) and thymidine incorporation at 24 h (B). One micromolar PGE_2 is used as positive control. $^*P < 0.05$ versus control (FCS alone). N = 3–4 wells per condition.

inhibition of DNA synthesis with the highest dose of the EP_4 agonist, the EP_4 antagonist could not block the effect of PGE_2 on GF proliferation, suggesting that EP_4 is not involved in this action. In contrast, the EP_2 agonist we used mimicked the effect of PGE_2 completely and convincingly. Thus we concluded that EP_2 mediates the anti-proliferative effect of PGE_2 in GFs, in agreement with other studies showing a role for this receptor in PGE_2-mediated inhibition of proliferation in lung [Huang et al., 2007] and liver [Koide et al., 2004] fibroblasts. In support of our conclusions, lung fibroblasts from mice treated with bleomycin (an agent which causes pulmonary fibrosis) lose their expression of EP_2 and become resistant to the anti-proliferative effect of PGE_2 and the same occurs in fibroblasts from EP_2 (−/−) mice [Moore et al., 2005]. These combined data point to EP_2 as the major receptor mediating the anti-mitogenic effect of PGE_2 in fibroblasts.

In many cell types, induction of proliferation by serum or growth factors, through their respective receptor tyrosine kinases (RTKs), is mediated via activation of the Mitogen-activated protein kinases

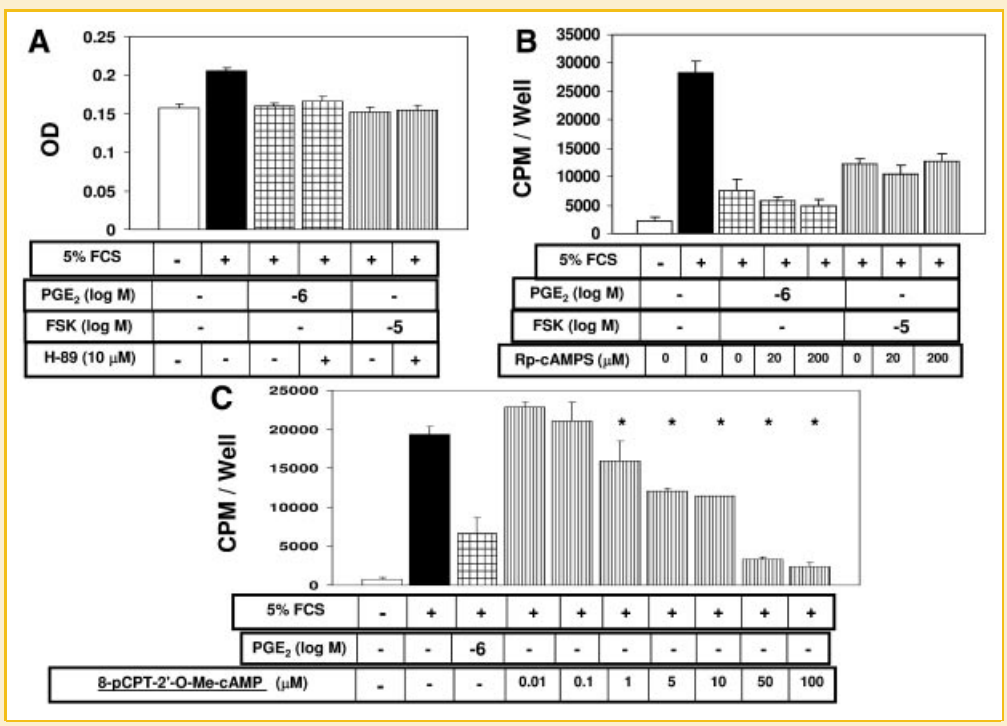

Fig. 6. The PKA inhibitors H-89 (A) and Rp-cAMPS (B) fail to abrogate the anti-mitogenic effect of 1 μM PGE_2 or 10 μM forskolin (FSK). In (A) cell number (at 48 h) is presented as OD and in (B), DNA synthesis (at 24 h) is presented as thymidine incorporation. C: The Epac activator (8-pCPT-2′-O-Me-cAMP) inhibits FCS-stimulated proliferation of hGFs (shown as thymidine incorporation at 24 h). One micromolar PGE_2 is used as positive control. $^*P < 0.05$ versus control (FCS alone). N = 3–4 wells per condition.

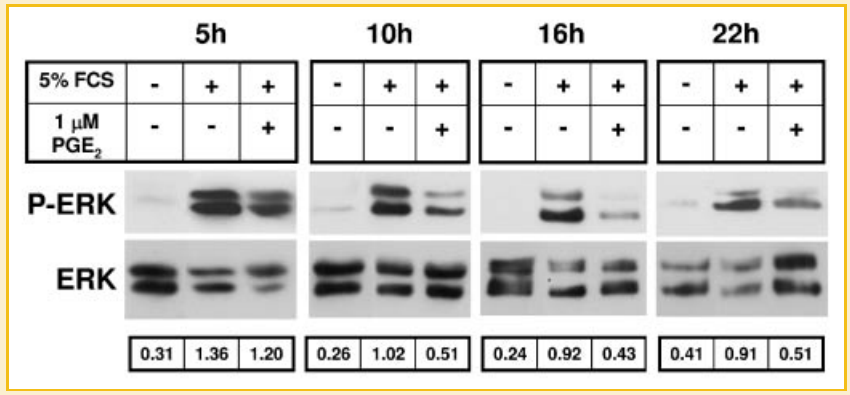

Fig. 7. Representative Western blotting analysis of the amount of phospho-ERK (p-ERK) and total ERK (ERK) in hGFs. Cells were incubated with serum-free medium or medium containing 5% FCS or 5%FCS+ 1 μM PGE_2 for 5–22 h. The ratio p-ERK/ERK derived by densitometry is provided under each lane.

(MAPK) cascade [Roux and Blenis, 2004]. The hallmark of the activation of this cascade is the phosphorylation of extracellular signal-regulated kinases (ERK) 1 and 2 and this was clearly shown in our data. ERK activation and its subsequent mitogenic signaling are under complex physiological control and one of the pathways known to inhibit it in many cell types is cAMP [Schmitt and Stork, 2001]. There are several possible ways in which elevated levels of cAMP can inhibit cell-cycle progression [reviewed in Stork and Schmitt, 2002; New and Wong, 2007]. The first and foremost mechanism is via inhibition of MAP kinase activity and ERK phosphorylation [Cook and McCormick, 1993] and the second is by affecting downstream targets independently of ERK, for example, inhibiting cell cycle-promoting cyclins (D for instance) or stimulating cell cycle inhibitors (p^{21Cip1} for instance). We clearly show here that PGE_2 treatment of hGFs reduces significantly the amount of phospho-ERK. Thus, although PGE_2 and serum transmit their opposite effects on GF proliferation via distinct (cAMP-dependent and cAMP-independent, respectively) post-receptor pathways, these signals converge at the level of phospho-ERK.

There are two well-described modes, through which elevated levels of cAMP can suppress ERK activation and proliferation: The first is by activation of PKA, which directly or indirectly suppresses the MAPK pathway [Burgering and Bos, 1995] and the second is by a PKA-independent activation of Epac, which is a guanine nucleotide exchange factor for the Ras-like small GTPases Rap1 and Rap2 [reviewed in Bos, 2003].

Some of the anti-mitogenic actions of cAMP were shown to require PKA activation. For instance, smooth muscle cell proliferation in vivo is inhibited by cAMP-PKA signaling [Indolfi et al., 1997]. Also, PDGF-induced ERK phosphorylation in arterial smooth muscle cells [Graves et al., 1993] and insulin-induced MAPK activity in adipocytes [Sevetson et al., 1993] are inhibited by the cAMP-PKA axis. In contrast, cAMP inhibition of the proliferation of retinal pigmented epithelial cells [Hecquet et al., 2002] or smooth muscle cells [Kassel et al., 2008] is PKA-independent. In terms of gingival fibroblasts, the only previously existing data were that the anti-mitogenic effect of PGE_2 on a GF cell line was not abrogated by HA1004, a PKA antagonist [Arai et al., 1998]. In agreement with these observations, we were unable to block the anti-mitogenic

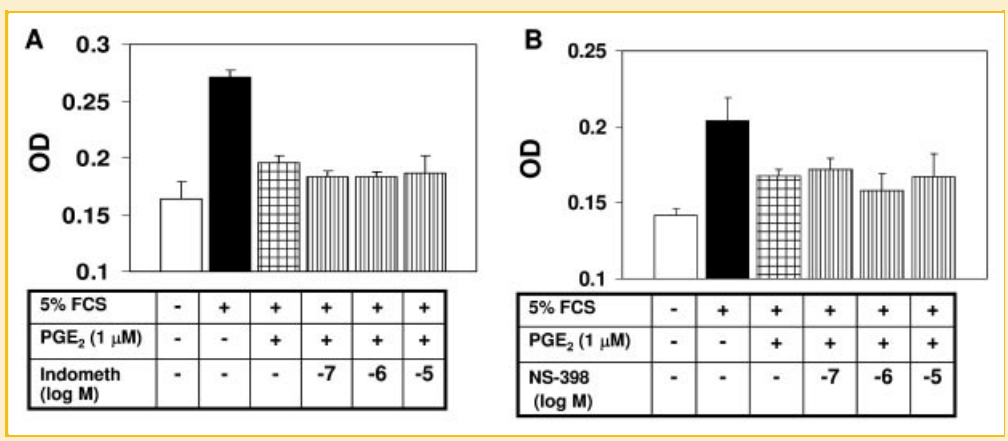

Fig. 8. The 2 COX-inhibitors (Indomethacin (indometh) in A and NS-398 in B) fail to prevent the anti-mitogenic effect of 1 μM PGE_2. Cell number is shown at 48 h as crystal violet OD. N = 3–4 wells per condition.

effect of PGE_2 with H-89 and Rp-cAMPS, two other PKA inhibitors. However, we found that the then-unavailable selective Epac activator 8-pCPT-2′-O-Me-cAMP mimicked completely the effect of PGE_2. Therefore we conclude that in primary human gingival fibroblasts, elevated levels of cAMP, subsequent to EP_2 ligation by PGE_2, activate the Epac signaling pathway and transmit an anti-mitogenic signal to the nucleus.

Finally, gingival fibroblasts are known to produce PGEs in response to various stimuli and express both of the COX isoforms [Gutiérrez-Venegas et al., 2005; Rausch-Fan et al., 2005]. Exogenous PGE_2 in vitro was reported to induce COX-2 expression in organ cultures of newborn mouse calvaria and in a cell line derived from this tissue (MC3T3-E1) [Pilbeam et al., 1995; Takahashi et al., 1994], raising the possibility that COX induction/PGE production is an autoamplification mechanism, activated by exogenous PGE_2. However, since 2 different COX inhibitors (Indomethacin and NS-398) failed to modulate hGF proliferation, our results indicate that the anti-mitogenic effect of PGE_2 in human gingival fibroblasts does not require COX-mediated production of prostanoids.

Prostanoids like PGE_2 are released in significant amounts in inflamed gingiva and may contribute to the inflammatory process and its sequelae in several levels [reviewed in Gemmel et al., 1997; Madianos et al., 2005; Noguchi and Ishikawa, 2007]. In addition to its participation in vasodilatation and increased capillary permeability, PGE_2 has been implicated for a long time in the induction of bone resorption, which is a prominent feature of periodontal disease [Page et al., 1997]. By inhibiting the proliferation of gingival fibroblasts, as described in this article, PGE_2 hampers the repair of the gingival connective tissue, which follows the inflammation-associated tissue destruction and cell death. In this respect TNFα, another cytokine strongly associated with the periodontal inflammation, has been reported by us and others to induce apoptosis of gingival [Zeldich et al., 2007b] and dermal [Alikhani et al., 2005] fibroblasts. Therefore both TNFα and PGE_2, the levels of which increase greatly in inflamed gingiva, contribute to the resulting gingival damage by targeting its major cellular component, gingival fibroblasts.

CONCLUSION

Prostaglandin E_2 inhibits the proliferation of human gingival fibroblasts via binding the EP_2 receptor, generation of cAMP, activation of Epac and suppression of ERK phosphorylation. Such action of this prostanoid, which is very abundant in inflamed gingiva, can hamper the repair of the gingival connective tissue, thus aggravating the tissue damage occurring in periodontal disease.

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