HHS Public Access

Author manuscript

J Dent Res. Author manuscript; available in PMC 2016 July 30.

Published in final edited form as:

J Dent Res. 2001 May ; 80(5): 1416–1420.

Low Magnitude of Tensile Strain Inhibits IL-1β-dependent Induction of Pro-inflammatory Cytokines and Induces Synthesis of IL-10 in Human Periodontal Ligament Cells *in vitro*

P. Long[1], **J. Hu**[2], **N. Piesco**[1], **M. Buckley**[2], and **S. Agarwal**[1,*]

[1]Department of Oral Medicine and Pathology, 589 Salk Hall, University of Pittsburgh School of Dental Medicine, 3501 Terrace Street, Pittsburgh, PA 15261-1964

[2]Department of Oral and Maxillofacial Surgery, University of Pittsburgh School of Dental Medicine, 3501 Terrace Street, Pittsburgh, PA 15261-1964

Abstract

Applied mechanical loading induces inflammation in the periodontal ligament (PDL). However, the mechanisms involved in bone deposition at tension sites in an inflammatory environment are not clear. Here, in an *in vitro* model system, we show that equibiaxial tensile strain of low magnitude (TENS) provokes potent anti-inflammatory signals in PDL cells. TENS inhibits IL-1β-induced synthesis of IL-1β, IL-6, and IL-8 by inhibiting their mRNA expression, and thus significantly suppresses the amplification of IL-1β-induced inflammatory responses in PDL cells. Additionally, as an anti-inflammatory signal, TENS induces IL-10 synthesis in the presence and absence of IL-1β. These observations are the first to demonstrate that TENS antagonizes IL-1β actions on PDL cells by (i) inhibiting IL-1β-induced transcriptional regulation of pro-inflammatory cytokines, and (ii) inducing synthesis of IL-10, which may post-transcriptionally suppress the synthesis of pro-inflammatory cytokines.

Keywords

periodontal ligament cells; tensile strain; cytokines

INTRODUCTION

During orthodontic tooth movement, periodontal ligament (PDL) cells respond to mechanical signals *via* synthesis of cytokines and growth factors, which regulate alveolar bone remodeling (Davidovitch, 1991; Shimuzu *et al*., 1998; Long *et al*., 2001). Interleukin (IL)-Iβ is implicated as one of the major cytokines synthesized by PDL cells in response to mechanical forces *in vivo* and *in vitro* (Saito *et al*., 1991; Burger *et al*., 1992; Shimizu *et al*., 1994, 1998; Klein-Neuland *et al*., 1995; Fermor *et al*., 1998; Noguchi *et al*., 1999). The presence of IL-1β, prostaglandin E_2 (PGE_2), and matrix metalloproteases (MMPs) in the periodontal tissue during tooth movement further implicates a role for these mediators in tissue resorption (Davidovitch, 1991; Grieve *et al*., 1994; Everts *et al*., 1996; Sinha and

*corresponding author, sagar@pitt.edu.

Nanda, 1996; Uematsu *et al.*, 1996; Nakaya *et al.*, 1997; Shimizu *et al.*, 1998; Chien *et al.*, 1999).

Interestingly, despite pro-inflammatory cytokine induction during tooth movement, bone is deposited at the tension sites. The intracellular mechanisms that favor osteogenesis over osteolysis under inflammatory conditions at the tension sites remain unknown. Paradoxically, the exposure of cells to mechanical strain is largely pro-inflammatory *in vitro* (Saito *et al.*, 1991; Shimizu *et al.*, 1994, 1998) and *in vivo* (Davidovitch, 1991; Saito *et al.*, 1991; Grieve *et al.*, 1994). Nevertheless, application of mechanical strain also initiates synthetic events such as production of collagen type I, fibronectin, alkaline phosphatase, and transforming growth factor (TGF)-β (Nakagawa *et al.*, 1994; Brady *et al.*, 1998; Howard *et al.*, 1998). Recently, we have shown that cyclic equibiaxial tensile strain (TENS) of a low magnitude is anti-inflammatory to PDL cells *in vitro* and inhibits IL-1β-induced cyclooxygenase mRNA expression and PGE_2 synthesis, as well as synthesis of MMP-1 and MMP-3 (Long *et al.*, 2001).

During inflammation, IL-1β autoregulates its own expression and induces IL-6 and IL-8 to amplify the cellular responses to external stimuli (Agarwal *et al.*, 1998; Manolagas, 1998; Rifas, 1999). Since TENS of low magnitude is anti-inflammatory (Long *et al.*, 2001), in this report we determined if the actions of TENS also down-regulate IL-1β-induced IL-1β, IL-6, and IL-8 production. Additionally, since IL-10 inhibits IL-1, IL-6, and IL-8 synthesis (Pertolani, 1999), we also investigated whether the anti-inflammatory activity of TENS also includes IL-10 induction.

MATERIALS & METHODS

Isolation and Characterization of PDL Cells

PDL cells were isolated from healthy erupted human third molars (University of Pittsburgh IRB approved), cultured in TCM (Basal Medium Eagle, 2 mM glutamine, Pen/Strep, GIBCO/BRL, Grand Island, NY, USA) and 10% calf serum (Hyclone Laboratories, Logan, UT, USA), cloned by limiting dilution, and used between the 6th and 20th passages. Osteoblast-like characteristics of PDL cell clones PL-442 and PL-150 (white females, ages 18 and 16 yrs), PL-484 (white male, age 22 yrs), and PL-75 (Asian male, age 16 yrs) were confirmed as described earlier (Agarwal *et al.*, 1998; Brady *et al.*, 1998).

Exposure of PDL Cells to TENS and IL-1β

Confluent PDL cells (5×10^5/well) were cultured in TCM on Bioflex II plates (Flexercell Inc., Hillsboro, NC, USA), washed, and incubated overnight with BME supplemented with serum replacement supplements, SRM-1 (Sigma Chemical Co., St. Louis, MO, USA). Cell monolayers were subjected to TENS at 0.005 Hz (Gassner *et al.*, 1999), where circumferential strain = 2π (change in radius)/2π (original radius) = (change in radius)/(original radius) = radial strain. The PDL cells were divided into four groups, *viz.*, untreated cells, cells treated with TENS, cells treated with IL-1β (1 ng/mL), and cells exposed to TENS and IL-1β (1 ng/mL; Genetech, LaJolla, CA, USA) simultaneously. The dose

response of the PDL cells to IL-1β showed that 1 ng/mL of IL-1β was sufficient to induce optimal COX-2 and PGE_2 expression (Long *et al.*, 2001).

Reverse Transcriptase/Polymerase Chain-reaction (RT/PCR)

RNA was extracted with a Qiagen RNA extraction kit (Santa Clarita, CA, USA). A total of 0.5 μg of RNA was reverse-transcribed and the cDNA amplified as described earlier (Xu *et al.*, 2000), with 30 cycles of amplification at 40 sec at 94°C, 40 sec at 62°C, and 60 sec at 72°C. The sequence of human primers used was as follows (Masahiro *et al.*, 1991): GAPDH (548 bp) sense 5′-GGTGAAGGTCGGAGTCAACGG-3′ , antisensc 5′-GGTCATGAGTCTTCCACGAT-3′; TL-1β (388 bp) sense 5′-AAACGAATGAAGTGCTCCTTCAGC-3′, antisense 3′-ACCTCGTTGTTCACCACAAGAGGT-5′; TNF-α (325 bp) 5′-ATGAGCACTGAAAGCATGATC-3′, antisense 3′-TCACAGGGCAATGATCCCAAAGTAGACCTGCCC-5′; IL-6 (639 bp) sense 5′-ATGAACTCTTTCTCCACAAGC-3′, antisense 3′-GTCAGGTCGGACTTTTGAGAAG-5′; IL-8 (302 bp) sense 5′-ATGACTTCCAAGCTGGCCGTG-3′, antisense 3′-TTATGAATTCTCAGCCCTCTTCAAAAACTT-5′; and IL-10 (420 bp) sense 5′-ATGCCCCAAGCTGAGAACCAAGACCCA-3′, antisense 3′-TCTCAAGGGGCTGGGTCAGCTATCCCA-5′. The semi-quantitative analysis of PCR products was performed with a Fluor-S MultiImager (BioRad, Hercules, CA, USA).

IL-1 Receptor Analysis

For functional analysis of IL-1 receptors, PDL cells were treated with TENS for one hour prior to the addition of IL-1β. Thereafter, the expression of IL-1β-induced IL-1β mRNA was examined after 4 hrs by RT/PCR, and its synthesis after 24 hrs by ELISA. The IL-1β responsiveness of these PDL cells was compared with that of cells exposed to IL-1β without treatment of TENS, and with that of cells treated with IL-1β and TENS.

Enzyme-linked Immunoassays

Total IL-1β, IL-6, IL-8 (Medgenix, Incstar, Stillwater, MN, USA), and IL-10 (Amersham Pharmacia, Piscataway , NJ, USA) in the culture supernatants was measured by ELISA kits according to the manufacturer's recommended protocols.

RESULTS

Effects of Various Magnitudes of TENS on Autoinduction of IL-1β

PDL cells exposed to 6%, 10%, or 15% TENS in the presence of TL-1β (1 ng/mL) for 24 hrs showed that 6% and 10% TENS markedly inhibited the IL-1β-induced IL-1β mRNA expression and synthesis. Contrarily, 15% TENS itself caused IL-1β gene induction, and augmented IL-1β synthesis above that induced by IL-1β alone (Figs. 1A, 1B). Due to its anti-inflammatory actions, 6% TENS was considered as low-magnitude TENS, and due to its pro-inflammatory actions, 15% TENS was used as high-magnitude TENS. To examine the anti-inflammatory effects, we used 6% TENS in the subsequent experiments.

TENS Suppresses Autocrine Regulation of IL-1β

Since IL-1β is a potent regulator of osteoclasia and amplifies its actions by autocrine induction, we examined whether TENS inhibits IL-1β-induced IL-1β production. As shown in Fig. 2A, TENS effectively inhibited (63%) autocrine induction of IL-1β mRNA within 4 hrs. The inhibition was sustained during the ensuing 44 hrs, showing 83% and 94% reductions at 24 and 48 hrs, respectively. The expression of mRNA for IL-1β was paralleled by its synthesis (Fig. 2B). Cells exposed to IL-1β and TENS for 24 or 48 hrs showed, respectively, 67% and 71% inhibitions of IL-1β accumulation in the culture supernatants. In all experiments, untreated PDL cells and those exposed to TENS alone failed to exhibit IL-1β production.

TENS Suppresses Transient Expression of TNF-α mRNA

IL-1β induced TNF-α mRNA expression during the first 4 hrs of activation, and this induction was inhibited (45%) by TENS. However, after 4 hrs, TNF-α gene expression was neither induced by IL-1β nor was it affected by TENS (Fig. 2A). The presence of TNF-α protein was not observed in culture supernatants of PDL cells subjected to any of the treatment regimens (data not shown).

TENS Suppresses IL-1β-dependent IL-6 Synthesis

IL-6 plays a major role in bone resorption during inflammation (Agarwal *et al.*, 1998; Manolagas, 1998; Rifas, 1999). TENS dramatically inhibits IL-β-induced IL-6 mRNA expression within the initial 4 hrs; this inhibits is sustained during the ensuing 44 hrs (Fig. 2A). The inhibition of IL-1β-dependent IL-6 mRNA expression was paralleled by the inhibition of IL-6 accumulation in culture supernatants when assessed by ELISA (78% after 24 hrs and insignificant accumulation between 24 and 48 hrs; Fig. 2C). In these experiments, control PDL cells also did not exhibits IL-6 synthesis.

TENS Inhibits IL-1βinduced IL-8 Production

TENS inhibited IL-1β-induced IL-8 mRNA expression within 4 hrs (64%) of activation, and the inhibition was sustained during the ensuing 20 hrs (87% inhibition) and 44 hrs (< 99% inhibition; Figs. 2A, 2D). Significant synthesis of IL-8 (above 20 pg/mL) in response to IL-1β was not observed within 4 hrs of activation. Nevertheless, IL-1β-induced IL-8 accumulated in notable quantities (180 pg/mL) in the culture supernatants. This accumulation of IL-8 was significantly inhibited (78%: $p \leq 0.05$) by simultaneous exposure to TENS within 24 hrs of activation. We failed to observe additional accumulation of IL-8 after 48 hrs of incubation (Fig. 2D).

TENS Induces IL-10 Expression in PDL Cells

The possibility existed that the TENS-mediated anti-inflammatory effects on PDL cells may be coupled to synthesis of IL-10 (Pertolani, 1999). IL-10 mRNA is not constitutively expressed in PDL cells, nor does IL-1β induce its expression. However, cells exposed to TENS alone expressed significant levels of IL-10 mRNA during the initial 4 hrs, and this was sustained for the ensuing 48 hrs. More importantly, TENS abrogated the ability of IL-1β to inhibit IL-10 production and induced higher mRNA expression for IL-10 during the intial

4 hrs, approximately equal to that induced by TENS alone at later time points (Figs. 3a, 3B). The accumulation of IL-10 in culture supernatants was not significantly different in cells exposed to TENS alone or to IL-1β and TENS (Fig. 3C).

TENS Actions Do Not Involve Down-regulation of IL-1 Receptors

The above results raised the possibility that TENS may act *via* down-regulation of the IL-1β receptor. We determined the responsiveness of PDL cells to IL-1β following exposure to TENS. IL-1β-induced IL-1β mRNA expression and synthesis was roughly equivalent in cells treated either with TENS for 1 hr prior to the addition of IL-1β and in cells treated with IL-1β alone. As expected, co-exposure of cells to TENS and IL-1β resulted in the inhibition of IL-1β-induced IL-1β mRNA expression and synthesis (Figs. 4A, 4B).

DISCUSSION

Much of the prior work focusing on responses of PDL cells to mechanical strain of high magnitude showed that mechanical stimuli are pro-inflammatory and elicit IL-1 and PGE_2 (Davidovitch, 1991; Shimizu *et al.*, 1994, 1998; Nakaya, 1997). Nevertheless, in response to applied mechanical forces, bone is formed at tension sites *in vivo* (Davidovitch, 1991). How the bone is deposited at these sites in an inflammatory environment is not well-understood. In an effort to understand such mechanisms *in vitro*, we have used: (i) PDL cells, because they have an osteoblast-like phenotype, respond to mechanical stimuli, and form bone at tension sites; (ii) TENS, because it closely mimics the strain to which PDL is subjected during tooth movement (Robersts *et al.*, 1992); and (iii) IL-1β, because it is implicated as a major mediator in stress-induced inflammation (Davidovitch, 1991; Grieve *et al.*, 1994).

PDL cells express IL-1β in response to activation by IL-1β (Shimizu *et al.*, 1994, 1998; Agarwal *et al.*, 1998; Noguchi *et al.*, 1999). TENS of low magnitude (6%) significantly inhibits autoregulation of IL-1β by down-regulating its mRNA expression within the first few hours of activation. This suggests that TENS inhibits the initial actions of IL-1β upstream of mRNA transcription. By inhibiting expression of IL-1β, TENS may drastically reduce the amplification of IL-1β-dependent immune responses (Klein-Neuland *et al.*, 1995; Manolagas, 1998; Rifas, 1999). Exposure to IL-1β was a prerequisite for the observed anti-inflammatory actions of TENS on PDL cells. More importantly, *in vitro,* only low-magnitude TENS elicited anti-inflammatory properties, whereas higher magnitudes of strain (15-18%) are inflammatory and induce IL-1β synthesis in PDL cells (Shimizu *et al.*, 1994, 1998; Klein-Neuland *et al.*, 1995). Whether low levels of mechanical loading exert anti-inflammatory effects *in vivo* is not yet clear. It is tempting to speculate that this may be case, because, during orthodontic treatment, low levels of mechanical loading lead to bone deposition at tension sites, whereas higher loading to accelerate tooth movement results in alveolar resorption with minimal bone deposition (Davidovitch, 1991; Sinha and Nanda, 1996).

IL-1β is an upstream cytokine that induces downstream cytokines such as TNF-α, which acts similarly to IL-1β, IL-6, which has a more direct role in osteoclast formation, differentiation, and activation (Manolagas, 1998; Rifas, 1999), and IL-8, which amplifies immune responses by attracting and activating immune cells to produce pro-inflammatory mediators (Dinarello,

1996). Interestingly. IL-1β does not induce TNF-α synthesis in PDL cells. However. by inhibiting IL-6 and IL-8 synthesis. TENS may indirectly up-regulate osteogenesis by minimizing the amplification of immune mediators and thus down-regulating osteoclastic differentiation at the tension sites.

IL-10 is a potent anti-inflammatory cytokine that inhibits the production of pro-inflammatory cytokines like IL-1β, TNF-α, IL-6, and IL-8 (Pertolani , 1999). TENS induces IL-10 mRNA expression in PDL cells independently, and in the presence of IL-1β it augments IL-10 synthesis by abrogating the ability of IL-Iβ to inhibit IL-10 synthesis (Pertolani, 1998). We have observed that TENS inhibits pro-inflammatory gene expression within the first 4 hrs, but the synthesis of IL-10 was not observed during the first 4 hrs, so it is unlikely that the actions or TENS are solely mediated *via* synthesis of IL-10. However, after the initiation of IL-10 synthesis, it is likely that the inhibition of pro-inflammatory cytokine induction is mediated either by IL-10 alone, or by both TENS and IL-10.

Due to the requirement of very few receptors *per* cell for IL-1β responsiveness, and due to their modulation in the presence of IL-1β, functional assay was considered more adequate for the possible TENS-mediated down-regulation of IL-1 receptors (Dinarello, 1996). Since pre-exposure of PDL cells to TENS did not affect IL-1β responsiveness, TENS does not impair the ability of PDL cells to respond to IL-1β at the receptor level, and IL-1β-receptor down-regulation may not be the key mechanism of TENS actions.

In summary, the present findings are the first to show that TENS of a low magnitude inhibits IL-1β-induced pro-inflammatory cytokine expression in PDL cells. Specifically, anti-inflammatory signals generated by TENS appear to provoke two notable changes: (i) inhibition of gene expression for pro-inflommatory cytokines *via* interception of the signal transduction cascade of IL-1β upstream of mRNA transciption, and (ii) induction of IL-10, which may facilitate TENS actions *via* inhibition of pro-inflammatory cytokine gene expression. On the other hand, in the absence of IL-β, tensile strain is known to induce synthesis of alkaline phosphatase and transforming growth factor (TGF)-β1 in PDL cells (Brady *et al.*, 1998). Therefore, it is tempting to speculate that, by inhibiting pro-inflammatory actions of IL-1β on PDL cells and inducing osteogenic proteins in PDL cells (Long *et al.*, 2001), TENS may manifest potential for bone induction in the local inflammatory environment. At present, intracellular signals generated by TENS that mediate its actions are not known, but once delineated, these pathways may explain the molecular basis of bone synthesis at tension sites during orthodontic tooth movement.

ACKNOWLEDGMENTS

The authors express their thanks to Mr. H.H. Langkamp for his critical comments on the manuscript. This work was supported by NIH grant #1R15DE12976, and by an Oral and Maxillofacial Surgery Foundation Research Grant.

REFERENCES

Agarwal S, Chandra CS, Piesco NP, Langkamp HH, Bowen L, Baran C. Regulation of periodontal ligament cell functions by Interleukin-1β. Infect Immun. 1998; 66:932–937. [PubMed: 9488378]

Brady TA, Piesco NP, Buckley MJ, Langkamp HH, Bowen LL, Agarwal S. Autoregulation of periodontal ligament cell phenotype and functions by transforming growth factor-β1. J Dent Res. 1998; 77:1779–1790. [PubMed: 9786634]

Burger EH, Klein-Neuland J, Veldhuijzen JP. Mechanical stress and osteogenesis *in vitro*. J Bone Miner Res. 1992; 7:397S–401S.

Chien HH, Lin WL, Cho MI. Interleukin-I beta-induced release of matrix proteins into culture media causes inhibition of mineralization of nodules formed by periodontal ligament cells *in vitro*. CalcifTissue Int. 1999; 64:402–413.

Davidovitch Z. Tooth movement. Crit Rev Oral Biol Med. 1991; 2:411–450. [PubMed: 1742417]

Dinarello CA. Biologic basis for interleukin-I in disease. Blood. 1996; 87:2095–2350. [PubMed: 8630372]

Everts, V.; Creemers, LB.; Docherty, JP.; Beertsen, W. Intracellular versus extracellular digestion of collagen. In: Davidovitch, Z.; Norton, LA., editors. Biological mechanisms of tooth movement and craniofacial adaptation. Harvard Society for the Advancement of Orthodontics; Boston, MA: 1996. p. 309-316.

Fermor B, Gundle R, Evans M, Emerton M, Pocock A, Murray D. Primary human osteoblast proliferation and prostaglandin E2 release in response to mechanical strain *in vitro*. Bone. 1998; 22:637–643. [PubMed: 9626402]

Gassner R, Buckley MJ, Georgescu H, Studer R, Stefanovich-Racic M, Piesco NP, et al. Cyclic tensile stress exerts anti-inflammatory actions on PDL cells by inhibiting inducible nitric oxide synthase. J Immunol. 1999; 163:2187–2193. [PubMed: 10438960]

Grieve WG, Johnson GK, Moore RN, Reinhart RA, DuBois LM. Prostaglandin E2 and interleukin-I levels in gingival crevicular fluid during human orthodontic tooth movement. Am J Orthod Dentofac Orthop. 1994; 105:369–374.

Howard PS, Kucich U, Taliwal R, Korostoff JM. Mechanical forces alter extracellular matrix synthesis by human periodontal ligament fibroblasts. J Periodontal Res. 1998; 33:500–508. [PubMed: 9879524]

Klein-Neuland J, Van der Plas A, Semeins CM, Ajubi NE, Frangos JA, Bueger EH. Sensitivity of osteocytes to biomechanical stress *in vitro*. FASEB J. 1995; 9:441–445. [PubMed: 7896017]

Long P, Liu F, Piesco NP, Buckley MJ, Kapur R, Agarwal S. Signalling by mechanical strain involves transcriptional regulation of proinflarnmatory gene induction in human periodontal ligament cells *in vitro*. Bone. 2001 (manuscript in review).

Manolagas SC. The role of IL-6 type cytokines and their receptors in bone. Ann NY Acad Sci. 1998; 840:194–204. [PubMed: 9629251]

Masahiro Y, Uyemura K, Deans RJ, Weinberg K, Rea TH, Bloom B, et al. Defining protective responses to pathogens: cytokine profiles in leprosy lesions. Science. 1991; 254:277–279. [PubMed: 1925582]

Nakagawa M, Kukita T, Nakashima A, Kurisu K. Expression pf the type I collagen gene in rat periodontal ligament during tooth movement as revealed by in situ hybridization. Arch Oral Biol. 1994; 39:289–294. [PubMed: 8024492]

Nakaya H, Oates TW, Hoang AM, Kamoi K, Cochran DL. Effects of interleukin-I beta on matrix metalloproteinase-3 levels in human periodontal ligament cells. J Periodontol. 1997; 68:517–523. [PubMed: 9203094]

Noguchi K, Shitashige M, Ishikawa I. Involvement of cyclooxygenase-2 in interleukinlalpha-induced prostaglandin production by human periodontal ligament cells. J Periodontal. 1999; 70:902–908.

Pertolani M. Interleukin-10: an anti-inflammatory cytokine with therapeutic potential. Clin Exp Allergy. 1999; 29:1164–1171. [PubMed: 10469024]

Rifas L. Bone and cytokines: beyond IL-1, IL-6 and TNF-alpha. CalcifTissue Int. 1999; 64:1–7.

Roberts, WE.; Garrett, LP.; Katona, TR. Principles of orthodontic biomechanics: metabolic and control mechanisms. In: Carlson, DS.; Goldstein, SA., editors. Bone biodynamics in orthodontic and orthopedic treatment. University of Michigan Press; Ann Arbor, MI: 1992. p. 189-255.

Saito M, Saito S, Ngan PW, Shanfeld J, Davidovitch Z. Interleukin-I beta and prostaglandin E are involved in the response of periodontal cells to mechanical stress *in vivo* and *in vitro*. Am J Orthod Dentofac Orthop. 1991; 99:226–240.

Shimizu N, Yamaguchi M, Goseki T, Ozawa Y, Saito K, Takiguchi H, et al. Cyclic-tension force stimulates interleukin-1 beta production by human periodontal ligament cells. J Periodontal Res. 1994; 29:328–333. [PubMed: 7799213]

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 Periodontal. 1998; 69:670–677.

Sinha, PK.; Nanda, RS. The relationship between orthodontic tooth movement and root resorption in PGE2 and non-PGE2 treated sides in a rat model. In: Davidovitch, Z.; Norton, LA., editors. Biological mechanisms of tooth movement and craniofacial adaptation. Harvard Society for the Advancement of Orthodontics; Boston, MA: 1996. p. 337-348.

Uematsu, S.; Mogi, M.; Deguchi, T. Cytokine levels are elevated in gingival crevicular fluid during orthodontic tooth movement. In: Davidovitch, Z.; Norton, LA., editors. Biological mechanisms of tooth movement and craniofacial adaptation. Harvard Society for the Advancement of Orthodontics; Boston, MA: 1996. p. 223-232.

Xu Z, Buckley MJ, Evans CH, Agarwal S. Cyclic tensile strain acts as an antagonist of interleukin-1β actions in chondrocytes. J Immunol. 2000; 165:453–460. [PubMed: 10861084]

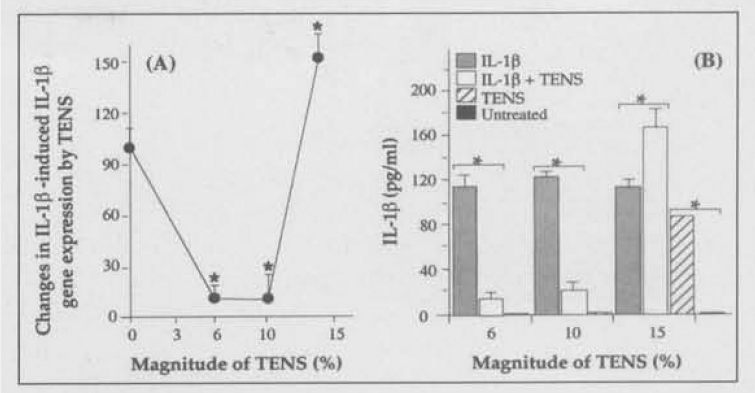

Figure 1.

Effects of various magnitudes of TENS on autoregulation of IL-1β in PDL cells. **(A)** Semiquantitative densitometric analysis of RT/PCR products for IL-1β following exposure of PDL cells to various magnitudes of TENS for 24 hrs. **(B)** Quantitative analysis of total IL-1β synthesis by ELISA in culture supernatants from PDL cells treated identically as in (A). The activating concentration of IL-1β (1 ng/mL) was subtracted from total IL-1β values. Results represent PL150 cells. Similar results were obtained from PL442, PL484, and PL75 cells. * indicates significant difference by ANOVA as compared with cells treated with IL-1β alone in (A) and between the two treatments under each horizontal bar in (B), calculated by ANOVA.

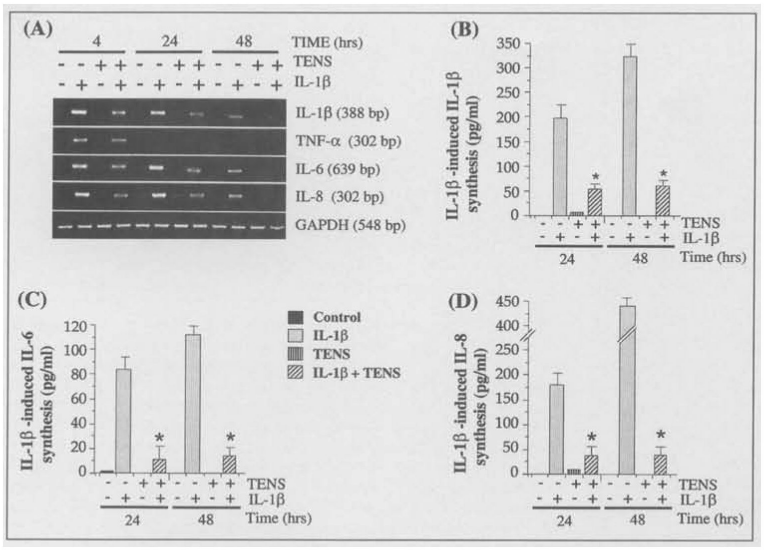

Figure 2.

(A) PDL cells were either untreated (−) or treated (+) with 6% TENS alone, IL-1β (1 ng/mL), or TENS (6%) and IL-1β for 4, 24, or 48 hrs. Subsequently, mRNA was extracted and analyzed by RT/PCR for IL-1β induced IL-1β, TNFα, IL-6, and IL-8. Semiquantitative densitometric analysis of ethidium-bromide-stained RT/PCR products showed a significant reduction ($p \leq 0.05$) in gene expression for each cytokine in cells treated with IL-1β and 6% TENS, in comparison with cells treated with IL-1β alone, at all time points. Analysis of **(B)** IL-1β, **(C)** IL-6, and **(D)** IL-8 synthesis in the culture supernatants of PDL cells as treated in (A) for 24 or 48 hrs. In (A), results are from PL150 cells performed in triplicate; similar results were obtained from PL442 and PL75 cells. In (B), (C), and (D), each point represents the mean and SEM of triplicate values from PL150 cells. Similar results were obtained from PL442 and PL75 cells. * indicates significant ($p \leq 0.05$) inhibition by ANOVYA as compared with cells treated with IL-1β alone.

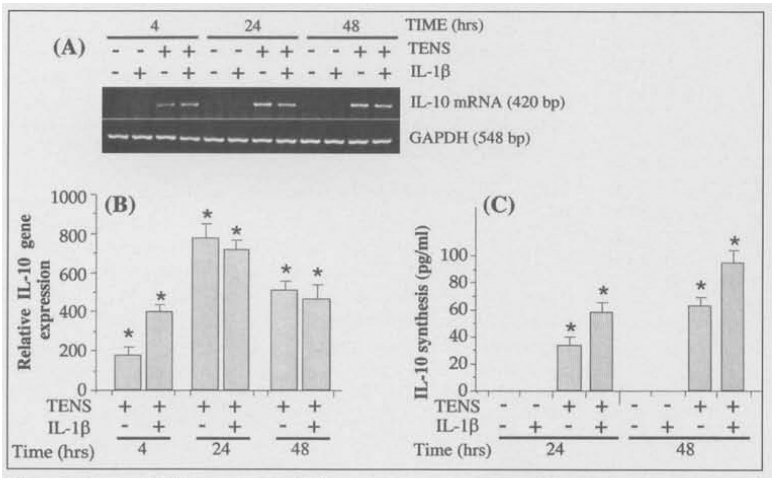

Figure 3.

Effects of TENS on IL-10 mRNA expression and synthesis. **(A)** Ethidium-bromide-stained RT/PCR products for IL-10 after treatment of PDL cells as described in the legend to Fig. 2A, for 4, 24, or 48 hrs. In comparison with untreated (−) controls and cells treated (+) with IL-β, there is a distinct expression of IL-10 mRNA in cells treated with TENS alone and with IL-1β and TENS. **(B)** Semiquantitative densitometric analysis of RT/PCR products shown in (A), showing relative expression of IL-10 mRNA in cells treated with TENS alone and those treated with TENS and IL-β. **(C)** IL-10 synthesis as measured by ELISA in the culture supernatants of PDL cells as treated in (A) for 24 or 48 hrs. In (A) and (B), results represent PL150 cells performed in triplicate; similar results were obtained with PL442 and PL75 cells, and in (C), each point represents the mean and SEM of triplicate values in PL150 cells. Similar results were obtained from PL442 and PL75 cells. * indicates significant ($p < 0.05$) induction of IL-10 as compared with cells treated with IL-β alone, calculated by ANOVA.

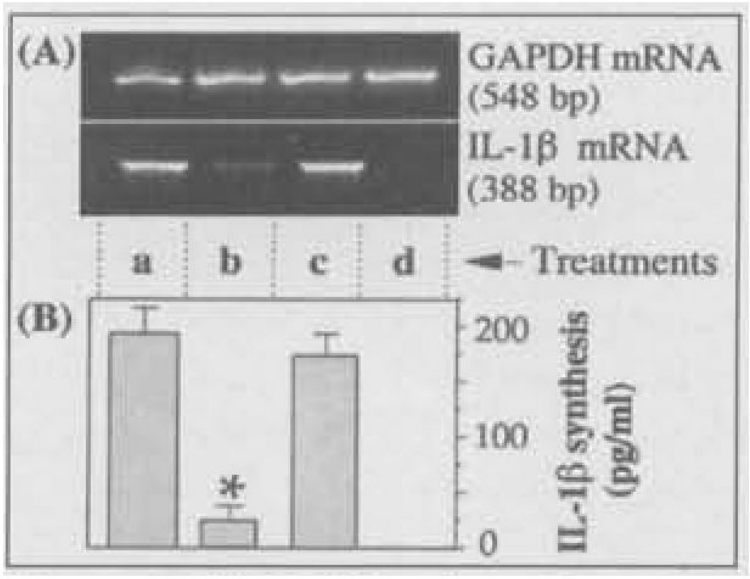

Figure 4.

(A) Ethidium-bromide-stained RT/PCR products for IL-1β in PL150 cells subjected to (a) IL-1β (1 ng/mL) alone, (b) IL-1β and TENS simultaneously, (c) pre-exposed to TENS for 1 hr prior to the addition of IL-1β (1 ng/mL), or (d) no treatment. Following a four-hour incubation, RNA was extracted and analyzed for IL-1β mRNA expression. The IL-1β;-induced IL-1β mRNA expression in cells pre-treated with TENS was similar to that in those treated with IL-1β alone. Similar results were obtained in PL75 cells. **(B)** Synthesis of IL-1β as measured by ELISA in cells treated for 24 hrs. Each point represents the mean and SEM of triplicate values. * indicates a significant ($p \leq 0.05$) difference in comparison with cells treated with IL-1β alone.