Inhibition of Gingival Collagenase Gene Expression by Dexamethasone (44293)

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Abstract. **Glucocorticoids are potent immunosuppressants shown to be effective in the treatment of inflammatory diseases. Reportedly, they work, in part, by inhibiting cytokines and other transcription factors including AP-1. In this study we investigated the mechanisms of efficient repression of collagenase gene expression by dexamethasone in the human gingival fibroblast. Northern analyses showed that IL-1–dependent collagenase mRNA production was significantly decreased in the presence of dexamethasone. The influence of dexamethasone on the transcription factor NF-κB, STAT3, and AP-1 was investigated by using the gel mobility shift assay with nuclear extracts prepared from the cells grown in the presence of dexamethasone. We observed that in addition to AP-1, binding of NF-κB and STAT3 to DNA was also decreased significantly. Additionally, dexamethasone induced the transcription of the IκB-α gene suggesting that in the presence of dexamethasone, NF-κB quickly reassociates with newly synthesized IκB-α and markedly reduces the amount of NF-κB. CAT transfection studies utilizing collagenase promoter demonstrated a dose-dependent transcriptional inhibition of IL-1–induced gingival collagenase gene expression by dexamethasone. These data reveal that collagenase gene expression can be regulated by the impairment of IL-1–stimulated NF-κB, STAT3, and AP-1 activities, and can highlight a possible molecular mechanism for the anti-inflammatory effects of glucocorticoids.** [P.S.E.B.M. 1998, Vol 218]

Increased levels of interleukin-1 (IL-1) and collagenase activity are reported in inflamed gingiva and in the gingival crevicular fluid (1, 2, 3). Extracellular collagen degradation is a central feature of inflammatory connective tissue lesions, and its level has been reported to be quite high in a number of diseases. Destructive lesions of joints, periodontium, and cornea are associated with secretion of collagenases and related matrix metalloproteinase (MMP) enzymes that can cleave and degrade collagens and connective tissue matrix molecules at physiological pH and temperature (4). A high level of collagenase activity can even be observed from the exudates obtained from affected sites in periodontal disease, and a direct correlationship has been established between neutrophilic collagenase activity and periodontal tissue destruction (5). Several *in vivo* studies demonstrated mRNA expressions of interstitial collagenase (MMP-1) by fibroblasts and macrophages in synovial tissue, and active collagenase has been identified in fluid from rheumatoid arthritis joints (6). Apart from MMP-1, which is the major enzyme responsible for tissue destruction, other collagenolytic enzymes, like MMP-8, gelatinase B, and MMP-9 are also secreted. They act along with myelooxidase and elastases. IL-1 is believed to contribute to the pathophysiology of periodontal diseases by activating collagenase gene expression. One of the earliest actions of the IL-1 signal is the induction of immediate early response genes that are important in the regulation of gingival collagenase expression. Recently, it was shown that the activator protein-1 (AP-1) family of transcription factors could play a major role in regulating collagenase gene expression. In addition, we have shown that induction of the nuclear factor, NF-κB, by IL-1 was associated with the gingival collagenase mRNA production (7). These immediate early responsive genes have been shown to be critical regulators of several genes involved in the immune and inflammatory

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responses (7, 8, 9). Thus, understanding the mechanisms of the suppression of gingival collagenase production by these key regulators of inflammation may be beneficial in preventing the soft tissue destruction associated with destructive periodontal diseases.

The modulation of IL-1 production may be useful for the control of various types of inflammatory reactions. Glucocorticoids (GCs) have been used as physiological inhibitors of inflammatory responses and immunosuppressive agents for decades, and mechanisms of their action is well understood (10). The present data will refine the molecular mechanism particularly in the context of collagenase gene expression and periodontal disease. GCs induce target genes through the glucocorticoid receptor (GR), a ligand-activated transcription factor. GCs repress gene expression through transcriptional interference between the activated GR and other transcription factors, most notably AP-1 and NF-κB through a cross-coupling mechanism (8). Recently, GCs have been shown to repress members of the NF-κB-rel transcription factor family by inducing the transcription of NF-κB inhibitor IκB-α (12, 13). These reports have also shown, however, that AP-1 and NF-κB activity could not account for the full spectrum of immunoregulatory genes affected by GCs. We initiated this study by extending our analyses of the transcriptional regulation of gingival collagenase gene expression by AP-1, NF-κB, and STAT3 in periodontal diseases. The STATs (signal transducers and activators of transcription) family of transcription factors have been shown to be activated by a number of cytokines and growth factors (14). STAT3 has been shown to have affinity for an element (SIE) in the c-*fos* promoter and may be in part responsible for activation of the c-*fos* gene during mitogen stimulation mediated by EGF and other growth factors (15, 16, 17). In this study, we analyzed the molecular mechanism of IL-1–dependent collagenase gene suppression by dexamethasone in human gingival fibroblasts and showed that dexamethasone could have an important association with the suppression of AP-1, NF-κB, and STAT3 activities.

Materials and Methods

Cell Culture. Discarded samples from gingivectomy procedures were collected in DMEM medium. The gingival tissues were minced and after trypsin (0.1% in HBS for 1 hr at 37°C) and collagenase (200 units/ml in complete medium for 20 min at 37°C) treatments, fibroblasts were allowed to grow to confluency. Human primary gingival fibroblasts were maintained in Eagle's medium (GIBCO Life Tech, Inc., Gaithersburg, MD) supplemented with 10% fetal bovine serum, glutamate, and pen/strep. These primary cells were serum-deprived overnight (~16 hr) by replacing the medium with medium containing only 0.05% fetal bovine serum. This allowed the cells to reach the same phase of cell division as well as reduce the background signal due to high serum concentration. The cells were treated with IL-1 at a final concentration of 100 ng/ml and/or dexamethasone at 1 μ*M* and 10 μ*M* concentrations.

RNA Preparation and Northern Blot Analysis. RNA was isolated by Ultraspec RNA isolation method as described by the manufacturer (Biotecx Lab, Inc., Houston, TX). RNA (10 μg) were electrophoresed on 1% agarose gel containing 2.2 *M* formaldehyde and blotted on to BA-S nitrocellulose (Schleicher and Schull, Inc., Keene, NH). The blots were baked at 80°C under vacuum and hybridized with radiolabeled probes in 10% dextran sulfate, 40% (v/v) formamide, 5 × SSC (1 × SSC = 0.15 *M* NaCl, 0.015 *M* sodium citrate), 5% Denhard's solution (1 × Denhardt's = 0.025% BSA, 0.025% Ficoll, and 0.025% Polyvinylpyrrolidone), 7 m*M* Tris-HCl (pH 7.4) buffer and 0.002% heat-denatured, sonicated salmon sperm DNA at 42°C for 16 hr. Blots were washed twice for 10 min each in 2 × SSC containing 0.1% SDS at room temperature and twice for 30 min each in 0.1% SSC containing 0.1% SDS at 50°C. The blot was then analyzed on the phosphoimager by autoradiography. To monitor equal loading and intactness of RNA, the same blot was deprobed by putting it in boiling 0.5% SDS solution for 30 min and rehybridized with a housekeeping gene, G3PDH, glyceraldehyde-3-phosphate dehydrogenase. The intensity of collagenase and IκB-α signals were corrected according to the signal of G3PDH. The results from three separate experiments were averaged, and intensities were plotted.

Gel Shift Analysis. The protein binding reactions were performed essentially as previously described (7). The consensus oligonucleotides were obtained from Promega as double-stranded pre-annealed DNA. The sequences for NF-κB, STAT3, and AP-1 oligos were 5′-ATG TGA**GGGGACTTTCCC**AGGC-3′, 5′-GATCC**TTCTGGGAAT**TCCTAGATC-3′, and 5′-CGCTTGA**TGACTCA**GCCGGAA-3′, respectively. Bold sequences denote the binding sites. The oligo nucleotides were end-labeled by polynucleotide kinase using γ-ATP^{32} by standard procedure. Then 10 μg of nuclear extracts were incubated with radiolabeled oligonucleotide (1 ng, 5–10,000 cpm) for 30 min at room temperature in 20 μl binding buffer (20 m*M* HEPES, pH 7.9/60 m*M* KCl/5 m*M* $MgCl_2$/0.2 m*M* EDTA/10% glycerol/0.5 m*M* PMSF/0.5 m*M* DTT/1% Nonidet P-40). After incubation, samples were electrophoresed through 5% polyacrylamide gels in 0.5 × TBE Buffer (44 m*M* Tris/44 m*M* Boric acid/1 m*M* EDTA). The gels were dried and exposed to Kodak x-ray film.

Transfection and CAT Assay (chloramphenicol acetyltransferase). The cells were split in 100-mm plates and transfected with 10 μg collagenase-CAT (chloramphenicol acetyltransferase) along with RSV-β-gal construct, 5 μg, using the calcium phosphate co-precipitation method for 4 hr as per manufacturer's protocol (Promaga Inc., Madison, WI). Glycerol shock was given to the cells by putting 15% of glycerol in Hank's Balanced Salt Solution for 2 min. The glycerol solution was removed, and the cells were washed three times with PBS. Subsequently, the cells were incubated in complete medium containing 10% fetal bovine serum. The cells were treated with dexamethasone (10 μm) for 24 hr and further with IL-1 (100 ng/ml) for 12 hr.

CAT Activity. Cells were washed twice with phosphate buffered saline (PBS) prior to being harvested in 1 ml PBS and stored in ice. After bench top centrifugation, pellets were resuspended in 200 μl of 0.25 *M* Tris-HCl (pH 7.5), and extracts were prepared by three cycles of freezing and thawing. Cellular debris was pelleted, and the supernatant was assayed for protein content by the Bradford method (30). To measure beta galactosidase activity, 10 μg of protein from each extract were taken, and fractions containing equal amounts of activity were incubated with 0.5 μCi [^{14}C] Chloramphenicol (57 mCi/mmol) and 0.53 m*M* butyryl CoA in 150 μl reaction volume containing 0.25 *M* Tris-HCl (pH 7.8). After incubation at 37°C for 60 min, the acetylated [^{14}C] Chloramphenicol was extracted with 2 volumes of ethyl acetate and dried under speed vac. The pellets were redissolved in 30 μl of ethyl acetate and analyzed by thin-layer chromatography in chloroform/methanol (97:3). The TLC plates were exposed with plates of phosphoimager for 2 to 3 hr, and quantitation was done using a Fuji Phosphoimager counter.

Results

Effect of Dexamethasone on Collagenase mRNA Production. Total RNA was extracted at different intervals from human gingival fibroblasts treated with IL-1 for 12 hr in the presence or absence of dexamethasone and subjected to Northern blot analysis. Figure 1A shows that in the presence of dexamethasone, IL-1–inducible collagenase mRNA production was significantly inhibited even at 1 μ*M* concentration. However, this inhibition was maximum at 10 μ*M* of dexamethasone, and the intensity of collagenase signal reached the level of the control signal. Quantitation of the autoradiograms by phosphoimager and correction of the values against G3PDH mRNA levels, revealed that dexamethasone-inhibited IL-1 induced collagenase mRNA production by approximately 90%, at 10 μ*M* concentration (Fig. 1B). These results suggest that dexamethasone could inhibit the IL-1–induced transcription of the collagenase gene.

Effect of Dexamethasone on Collagenase CAT Reporter Gene Expression. To determine if dexamethasone can inhibit the transactivation of the collagenase gene associated with its own promoter region, collagenase-CAT activities were determined in the construct containing 3.8 kb of 5′-flanking DNA of human collagenase gene linked to the CAT reporter gene. This construct contains the binding sites for AP-1, PEA3, and NF-κB as well as the collagenase TATA box and a transcriptional start site. When cells were transfected with the above vector (pcoll-CAT) and CAT assays were performed, dexamethasone inhibited the IL-1–induced CAT activities significantly (Fig. 2). Quantitation of the autoradiograms by phosphoimager revealed that there was about 10% acetylation in control cells without IL-1 or dexa due mainly to the endogenous transacting factors. Upon treating the cells with IL-1 alone, the percent CAT activity increased to about 60%, around six-

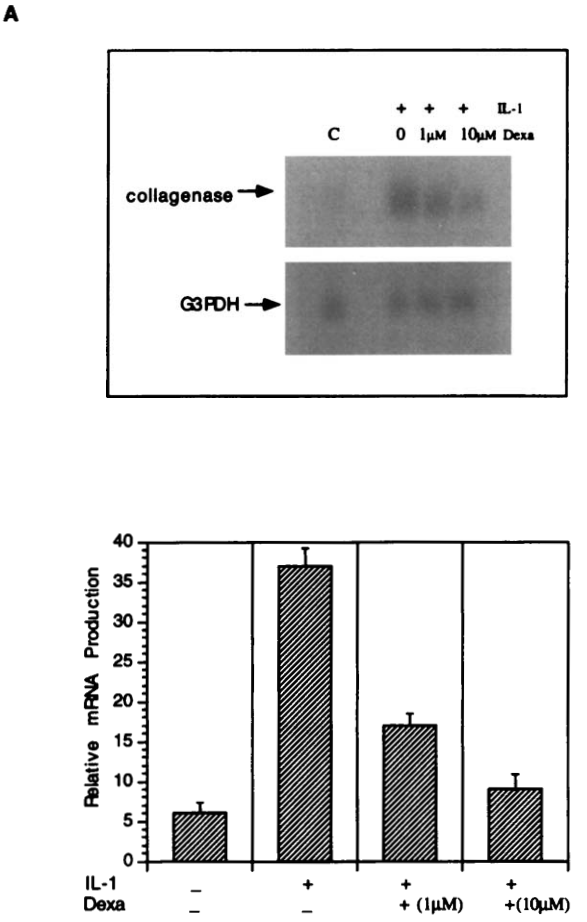

Figure 1. Northern blot analysis of the effect of dexamethasone on collagenase mRNA in human gingival fibroblasts. Quiescent human gingival fibroblasts were cultured alone or in the presence of IL-1 and both IL-1 and dexamethasone. [A] Total cellular RNA was extracted and analyzed by Northern blot analysis using ^{32}P-labeled c-DNA probes for collagenase and G3PDH. The resulting mean fold inhibition of three assays was determined with the aid of a Fuji phosphoimager. [B] Values were corrected according to the relative intensities of the G3PDH band. Label 'C' in A and B, denotes control RNA (i.e., without any treatment).

fold as compared to the basal activity. Interestingly, upon treating the cells with IL-1 in the presence of dexamethasone, the percent acetylation of chloramphenicol was reduced drastically to about 8%, which is below the basal level. This shows that dexamethasone inhibited transactivation of the collagenase gene by approximately 100%. This confirms that the effect of dexamethasone is mainly at the transcriptional level.

Induction of IκB-α Gene by Dexamethasone. Dexamethasone activates IκB-α gene transcription resulting in a rapid formation of IκBα-NF-κB complex which, in turn, could reduce the translocation of NF-κB into the nucleus. To determine if the GC-mediated gingival collagenase mRNA repression was accompanied with the increased IκB-α mRNA, Northern analyses were performed with the total RNA derived from gingival fibroblast cells treated with dexamethasone for increasing time periods. As shown in Figure 3, dexamethasone induces a significant increase in IκB-α mRNA, in a biphasic manner, reaching to its maxi-

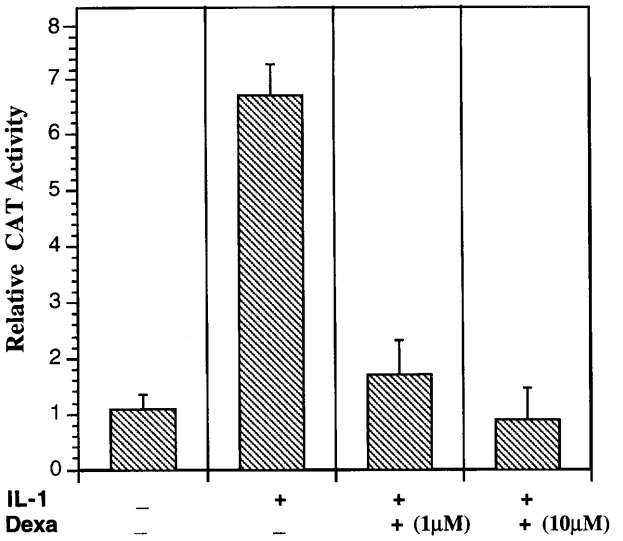

Figure 2. Analysis of inhibition of collagenase-CAT constructs of dexamethasone. Human gingival fibroblasts were transiently transfected with 10 μg of Coll-CAT3 and 5 μg of RSV beta galactosidase construct. Following glycerol shock, cells were allowed to recover for 16 hr in DMEM with 10% FCS. Cells were then treated with dexamethasone for 24 hr and then IL-1 for 12 hr. After treatment, cells were harvested, and CAT activities were determined. Values shown represent the mean percent acetylation of three separate determinations that were corrected for β-galactosidase activity.

mum in 1 and 8 hr. In agreement with others (12, 13), our data also suggest that GCs could also directly activate the IκB-α gene transcription in the gingival fibroblast cells, leading to a rapid turnover of IκBα protein and thus, restrict the entry of NF-κB into the nucleus. This suppression of NF-κB could potentially decrease the IL-1–induced collagenase gene expression.

Effect of Dexamethasone on the Activation of NF-κB, STAT3, and AP-1 Binding Activity. To examine the effect of dexamethasone on the binding of NF-κB, Stat3, and AP-1, required for the collagenase gene activation by IL-1, we performed gel shift assays using the sequences of individual nuclear factor binding sites. Nuclear proteins extracted from human gingival fibroblasts, which were treated with IL-1 in the presence or absence of dexamethasone, were subjected to gel shift assays. We observed that the IL-1 induced the formation of the NF-κB complex, which was maximum at 15 min. At 30 min, we still found the complex formation many fold less than at 15 min. In the presence of dexamethasone, we found a significant reduction in the complex formation during both periods of incubation (Fig. 4A). Recently, we have shown that IL-1 induces the STAT3 and is involved in collagenase gene regulation (unpublished). As shown in panel B, maximum induction of STAT3 protein takes place at 15 min of incubation, as in the case of NF-κB, and the latter is inhibited in the presence of dexamethasone. In the case of AP-1 (panel C), the maximum induction is observed at 15 min, as in the above two cases. In the presence of dexamethasone, the complex formation was inhibited more than five-fold. In contrary to NF-κB and STAT3, we find slightly more signal

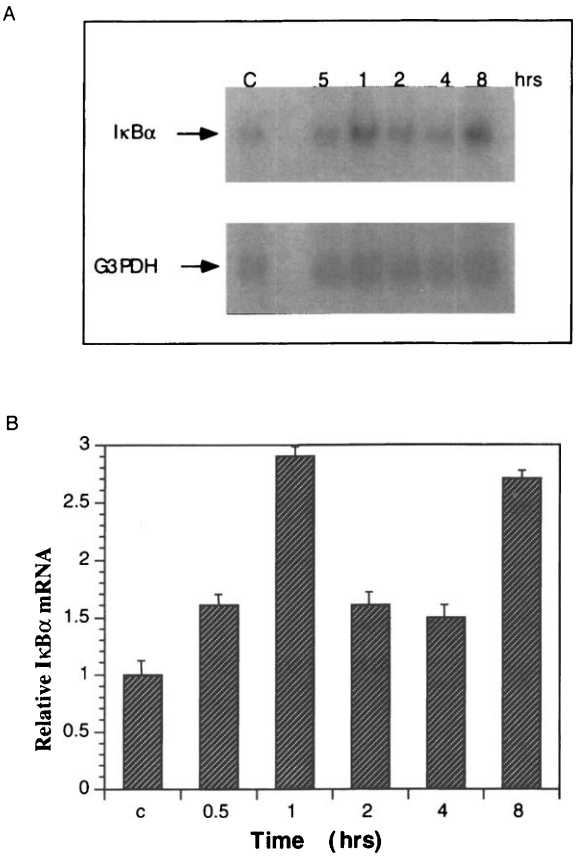

Figure 3. Time course of stimulation of IκB-α by dexamethasone. Gingival fibroblasts were cultured alone or in the presence of dexamethasone/IL-1 for the indicated time. [A] RNA was then prepared, and Northern blot analysis was performed. [B] The intensities of RNA signals were determined with the aid of a Fuji Phosphoimager, the average results from three experiments are shown. Values shown have been corrected with the intensities of the G3PDH housekeeping gene.

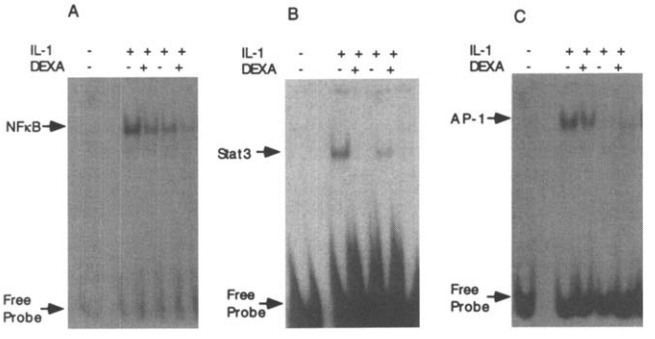

Figure 4. Characterization of the NF-κB, Stat3, and AP-1 DNA-binding activity inhibited in gingival fibroblasts by dexamethasone. Gingival cells were cultured to confluency before incubation with dexamethasone (10 μg/ml) and IL-1 (100 ng/ml). Nuclear extracts were prepared at 15 and 30 min of treatment. [A] NF-κB [B] STAT3, and [C] AP-1 DNA-binding activities were determined by electrophoretic mobility gel shift assay. Essentially identical results were obtained in three independent experiments. The presence and absence of dexa/IL-1 have been shown as + and – signs. Numbers indicate the time of induction with IL-1 in minutes. "C" denotes untreated control cells.

of AP-1 at 30 min of incubation in the presence of dexamethasone than we find in its absence. However, this signal is much less and may be due to some basal levels of these factors in the cells. Our results indicate that NF-κB, STAT3, and AP-1 are maximally induced within 15 min of IL-1 treatment, and this induction is downregulated by dexamethasone.

Discussion

Glucocorticoids are used as immunomodulators and inhibit the transcription of several cytokine genes, particularly IL-1, which is involved in the inflammatory process (11, 18). Despite the widespread use, the molecular mechanisms that underline their therapeutic effects are poorly understood (19) in the context of periodontal diseases. In this work, we studied the molecular mechanisms of repression of IL-1–induced collagenase gene in gingival fibroblast cells. It has been shown that glucocorticoids could target AP-1 and NF-κB transcription factors for their actions, but the molecular effects are still poorly understood. The inhibitory effect of dexamethasone is mediated through the glucocorticoid receptor. For NF-κB, which contains mostly P50 and P65 subunits (20), another possible mediator of the inhibitory action of dexamethasone is IκB-α (11, 12). The short lived IκB-α protein is encoded by the MAD-3 gene and its degradation is further enhanced by various NF-κB inducers (21, 22). The low level of protein stimulates its own synthesis in a feedback manner that terminates NF-κB activation. Inhibition of NF-κB activation has been suggested for many immunosuppressive and anti-inflammatory activities of glucocorticoids as NF-κB plays a central role in induction of a large number of important immunoregulatory genes including IL-1 (11, 23).

We report here, that dexamethasone could suppress the transcription of gingival collagenase mRNA. Consistent with these observations, we have also shown that transactivation of the collagenase-CAT reporter gene is significantly affected by dexamethasone. Addition of dexamethasone also induces steady state mRNA levels of IκB-α (Fig. 3). Other studies suggest that dexamethasone increases IκB-α protein level through an increase in the transcription of IκB-α gene (11–12, 24, 26). Our gingival fibroblast results suggest that the activities of NF-κB could be reduced due to the binding of newly synthesized IκB-α proteins with NF-κB and result in poor translocation into the nucleus. In addition to this mechanism, the newly synthesized IκBα may enter the nucleus and inhibit the preexisting NF-κB activity (27).

AP-1 has been found to be involved in the regulation of several inflammatory genes including collagenase. Dexamethasone is believed to down regulate AP-1 activity *via* an interaction between the DNA-binding domain of the ligand steroid receptor and the leucine-zipper peptide motif of c-*jun* (28). Recently, STAT3 factors have been shown to have affinity for an element (SIE) in the c-*fos* promoter and may be in part responsible for activation of the c-*fos* gene during mitogen stimulation mediated by EGF and other growth factors (16, 29). We have recently shown that transcription of the gingival collagenase gene could be modulated significantly by IL-1–induced AP-1 and STAT3 transcription factors (unpublished). The identification of AP-1, NF-κB and STAT3 as early factors in gingival fibroblasts provides additional clues as to the signal transduction pathways that are activated in the periodontal diseases within the first minutes of the IL-1 stimulation. We also checked whether the glucocorticoid interfered with the binding of AP-1 and STAT3 factors in the collagenase gene promoter and represented its activation. For this purpose, gel shift analyses were performed using the cell extracts of human gingival fibroblast cells treated with IL-1 in the presence or absence of dexamethasone. Our studies revealed that dexamethasone, besides NF-κB, also inhibited the binding activity of AP-1 and STAT3.

These results demonstrate that the inhibitory effect of glucocorticoids occurs through transcription and transactivation of the collagenase promoter. In addition, inhibition of AP-1, NF-κB, and STAT3 activity suggests that these sites are important not only for gingival collagenase induction by IL-1, but also for the inhibition of these responses by dexamethasone. Our results further show that glucocorticoids could inhibit gingival collagenase gene expression through different mechanisms and that this repression could have an important role in future studies and expression of a number of immunoregulatory genes.

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1. Matsuki Y, Yamamoto T, Hara K. Localization of interleukin-1 (IL-1) mRNA-expressing macrophages in human inflamed gingiva and IL-1 activity in gingival crevicular fluid. J Periodontal Res **28:**35–42, 1993.
2. Tewari DS, Qian Y, Tewari M, Pieringer J, Thornton RD, Taub R, Mochan E. Mechanistic features associated with induction of metalloproteinases in human gingival fibroblasts by interleukin-1. Arch Oral Biol **39:**657–664, 1994.
3. Tatakis DN. Interleukin-1 and bone metabolism: A review. J Periodontol **64:**416–431, 1993.
4. Harris ED, Krane SM. Collagenases (first of three parts). N Eng J Med **291:**557–661, 1974.
5. Lee W, Aitken S, Sodek J, McCulloch CAG. Evidence of a direct relationship between neutrophil collagenase activity and periodontal tissue destruction *in vivo:* Role of active enzyme in human periodontitis. J Periodontal Res **30:**23–33, 1995.
6. Harris ED Jr., Cohen GL, Krane SM. Synovial collagenase: Its presence in culture from joint disease etiology. Arthritis Rheum **34:**1085–1093, 1991.
7. Tewari M, Tuncay OC, Milchman A, Reddy PJ, Reddy CD, Cressman DE, Taub R, Newton RC, Tewari DS. Association of interleukin-1-induced NF kappa B DNA-binding activity with collagenase gene expression in human gingiva fibroblasts. Arch Oral Biol **41:**461–468, 1996.
8. Vincenti MP, Coon CI, Lee O, Brinckerhoff CE. Regulation of collagenase gene expression by IL-1 beta requires transcriptional and post-transcriptional mechanisms. Nucleic Acids Res **22:**4818–4827, 1994.

9. Vincenti MP, Coon CI, White LA, Barchowsky A, Brinckerhoff CE. *src*-Related tyrosine kinases regulate transcriptional activation of the interstitial collagenase gene, MMP-1, in interleukin-1-stimulated synovial fibroblasts. Arthritis Rheum **39:**574–582, 1996.
10. Siebenlist U. NF kappa B/I kappa B proteins. Their role in cell growth, differentiation, and development. Biochim Biophys Acta **1332:**R7–13, 1997.
11. Cato AC, Wade E. Molecular mechanisms of anti-inflammatory action of glucocorticoids. Bioessays **18:**371–378, 1996.
12. Auphan N, DiDonato JA, Rosette C, Helmberg A, Karin M. Immunosuppression by glucocorticoids: Inhibition of NF-kappa B activity through induction of I-kappa B synthesis. Science **270:**286–290, 1995.
13. Scheinman RI, Cogswell PC, Lofquist AK, Baldwin AS Jr. Role of transcriptional activation of I-kappa B alpha in mediation of immunosuppression by glucocorticoids. Science **270:**283–286, 1995.
14. Ihle JN. STATs and MAPKs: Obligate or opportunistic partners in signaling. Bioessays **18:**95–98, 1996.
15. Ihle JN. STATs: Signal transducers and activators of transcription. Cell **84:**331–334, 1996.
16. Darnell JE Jr. Reflections on STAT3, STAT5, and STAT6 as fat STATs. Proc Natl Acad Sci USA **93:**6221–6224, 1996.
17. Kapstein A, PailSengupta TK, Schmitt EM, Ivashkiv LB. Inhibition of cytokines and JAK-STAT activation by distinct signaling pathways. Proc Natl Acad Sci USA **93:**9499–9504, 1996.
18. Scheinman RI, Gualberto A, Jewell CM, Cidlowski JA, Baldwin AS Jr. Characterization of mechanisms involved in transrepression of NF-kappa B by activated glucocorticoid receptors. Mol Cell Biol **15:**943–953, 1995.
19. Krane SM. Some molecular mechanisms of glucocorticoid action. Br J Rheumatol **32:**3–5, 1993.
20. Boumpas DT. A novel action of glucocorticoids: NF-kappa B inhibition. Br J Rheumatol **35:**709–710, 1996.
21. Whiteside ST, Ernst MK, LeBail O, Laurent-Winter C, Rice N, Israel AN. C-terminal sequences control degradation of MAD3/I kappa B alpha in response to inducers of NF-kappa B activity. Mol Cell Biol **15:**5339–5345, 1995.
22. Le Bail O, Schmidt-Ullrich R, Israel A. Promoter analysis of the gene encoding the I kappa B-alpha/MAD3 inhibitor of NF-kappa B: Positive regulation by members of the rel/NF-kappa B family. EMBO J **12:**5043–5049, 1993.
23. Watanabe T, Tan N, Saiki Y, Makisumi T, Nakamura S. Possible involvement of glucocorticoids in the modulation of interleukin-1-induced cardiovascular responses in rats. J Physiol **491:**231–239, 1996.
24. Beauparlant P, Hiscott J. Biological and biochemical inhibitors of the NF-kappa B/Rel proteins and cytokine synthesis. Cytokine Growth Factor Rev **7:**175–190, 1996.
25. DiDonato JA, Saatcioglu F, Karin M. Molecular mechanisms of immunosuppression and anti-inflammatory activities by glucocorticoids. Am J Respir Crit Care Med **154:**511–515, 1996.
26. Scheinman RI, Gualberto A, Jewell CM, Cidlowski JA, Baldwin AS Jr. Characterization of mechanisms involved in transrepression of NF-kappa B by activated glucocorticoid receptors. Mol Cell Biol **15:**943–953, 1995.
27. Kathy T, Menie M, Dimitris T. Distinct functional properties of IκB-α and IκBb., Mol Cell Biol **17:**5386–5399, 1997.
28. Adcock IM, Brown CR, Shirasaki H, Barnes PJ. Effects of dexamethasone on cytokine and phorbol ester stimulated c-*fos* and c-*jun* DNA binding and gene expression in human lung. Eur Respir J **7:**2117–2123, 1994.
29. Tian SS, Tapley P, Sincich C, Stein RB, Rosen J, Lamb P. Multiple signaling pathways induced by granulocyte colony-stimulating factor involving activation of JAKs, STAT5, and/or STAT3 are required for regulation of three distinct classes of immediate early genes. Blood **88:**4435–4444, 1996.
30. Bradford MM. A refined and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. Anal Biochem **72:**248, 1976.