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MAPKs, activator protein-1 and nuclear factor-κB mediate production of interleukin-1β-stimulated cytokines, prostaglandin E_2 and MMP-1 in human periodontal ligament cells

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Background and Objective: Determination of the interleukin-1 (IL-1) signaling cascades that lead to the production of various inflammatory mediators and catabolic factors may clarify attractive targets for therapeutic intervention for periodontitis. We comprehensively assessed the involvement of MAPKs, activator protein-1 (AP-1) and nuclear factor-κB (NF-κB) in IL-1β-induced production of interleukin-6 (IL-6), interleukin-8 (IL-8), prostaglandin E_2 (PGE_2) and MMP-1 in human periodontal ligament cells.

Material and Methods: Human periodontal ligament cells were pretreated with an inhibitor for each of the MAPKs or NF-κB and subsequently treated with IL-1β. Following treatment, phosphorylation of three types of MAPK (ERK, p38 MAPK and c-Jun N-terminal kinase), IκB kinase (IKK) α/β/γ and IκB-α, as well as the DNA binding activity of AP-1 and NF-κB and the production of IL-6, IL-8, PGE_2 and MMP-1, were determined by western blotting, a gel mobility shift assay and ELISA, respectively.

Results: The three MAPKs, simultaneously activated by IL-1β, mediated the subsequent DNA binding of AP-1 at various magnitudes, while IKKα/β/γ, IκB-α and NF-κB were also involved in the IL-1 signaling cascade. Furthermore, IL-1β stimulated the production of IL-6, IL-8, PGE_2 and MMP-1 via activation of the three MAPKs and NF-κB, because inhibitors of these significantly suppressed the IL-1β-stimulated production of these factors.

Conclusion: Our results strongly suggest that MAPK, AP-1 and NF-κB mediate the IL-1β-stimulated synthesis of IL-6, IL-8, PGE_2 and MMP-1 in human periodontal ligament cells. Therefore, inhibition of activation of MAPK, AP-1 and/or NF-κB may lead to therapeutic effects on progression of periodontitis.

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The involvement of interleukin-1 (IL-1), a multifunctional inflammatory cytokine, in the pathogenesis of periodontitis has been suspected for a number of years (1), while clinical studies have also demonstrated a correlation between this cytokine and periodontitis (2,3). Furthermore, the immunopathological activities attributed to IL-1 are due to its ability to induce various inflammatory mediators and catabolic factors, such as other cytokines, prostanoids, nitric oxide synthase and MMPs, in various types of host cells.

It is well known that cellular responses to IL-1 are mediated by cascades of intracellular signaling events, including activation of MAPKs/activator protein-1 (AP-1) and inhibitor of KB (IκB) kinases (IKKs)/IκB/nuclear factor-κB (NF-κB). Interleukin-1 induces recruitment of tumor necrosis factor (TNF) receptor-associated factor 6, which leads to activation of NF-κB-inducing kinase (NIK). This, in turn, activates IKKs by serine phosphorylation, which subsequently phosphorylate IκB proteins, resulting in the degradation of IκB through a ubiquitin–proteasome-dependent mechanism. Proteolysis of IκB releases NF-κB and allows it to translocate to the nucleus, where it activates the transcription of specific target genes. In contrast, AP-1 is a critical regulator of several genes involved in inflammatory responses induced by proinflammatory cytokines, including IL-1. Interleukin-1 also activates the principal MAPK family members, including ERK, p38 MAPK and c-Jun N-terminal kinase (JNK), in responsive cell types, while those three MAPKs subsequently activate a number of transcriptional factors, including AP-1. Therefore, MAPK/AP-1 and NF-κB may be closely involved in periodontal inflammation attributed to IL-1.

Human periodontal ligament (HPL) cells may play important roles in local inflammatory and immune responses, as well as in tissue destruction in inflamed periodontium, as they are stimulated to produce various immunophathological mediators, such as interleukin-6 (IL-6), interleukin-8 (IL-8) and prostaglandin E_2 (PGE_2), along with collagenolytic enzymes such as MMPs, in response to IL-1 (4–7). In addition, constitutively expressed IL-1β mediates mechanical stress-induced IL-8 expression in HPL cells (8). Therefore, determination of IL-1 signaling cascades that lead to production of these factors may clarify attractive targets for use in therapeutic intervention for destructive periodontal disease. The roles of MAPK/AP-1 and NF-κB in the synthesis of IL-1-induced inflammatory mediators and catabolic factors have been investigated using several types of fibroblasts; however, they remain controversial. In the present study, we comprehensively determined the involvement of MAPK/AP-1 and NF-κB in IL-1β-induced production of IL-6, IL-8, PGE_2 and MMP-1 by HPL cells.

Material and methods

Cell culture

The HPL cells were collected by enzymatic digestion from HPL tissues, which were located in the mid-third of premolar roots extracted from three adult patients (an 18-year-old man; and two women, 20 and 22 years of age) with clinically healthy periodontium. All patients were duly informed of the nature and extent of the study, and their informed consent was obtained according to the Declaration of Helsinki. The protocol for the present study was approved by the Ethics Committee of Clinical Study of Showa University School of Dentistry (Tokyo, Japan). Human periodontal ligament tissues located in the mid-third of premolar roots were incubated for 1 h at 37°C in 0.2% collagenase (Wako Pure Chemical Industries Ltd, Saitama, Japan) in α-minimal essential medium (α-MEM; Flow Laboratories, McLean, VA, USA) supplemented with 100 U/mL of penicillin and 100 μg/mL of streptomycin. Cell suspensions were collected, filtered with a cell strainer, and centrifuged at 1000*g* for 10 min at 4°C. Each cell pellet was resuspended in medium with 10% fetal bovine serum (Gibco BRL, Grand Island, NY, USA) and cultured at 37°C in 95% air and 5% CO_2 until confluent cell monolayers were formed. Typical HPL cells prepared from samples from each of the three patients were designated as HPL-1, -2 and -3 cells, respectively, and used at passages 3 and 4 in all of the experiments.

The HPL cells were cultured for 72 h until confluent. Prior to treatment, the cells were pre-incubated for 12 h in medium containing 1% fetal bovine serum, then treated with recombinant human IL-1β (2.5 ng/mL; Genzyme-Techne, Minneapolis, MN, USA) for between 10 min and 48 h. Some cells were pretreated for 30 min or 1 h with PD098059 (1 μM; Sigma Chemical Co., St Louis, MO, USA), U0126 (1 μM; Promega Co., Madison, WI, USA), SB203580 (1 μM; Sigma), SP600125 (10 μM; Calbiochem, San Diego, CA, USA), pyrrolidine dithiocarbamate (PDTC; 2 μM; Sigma), *N*-acetyl-L-cysteine (NAC; 0.1 μM; Sigma), gliotoxin (1 μM; Calbiochem) or carbobenzoxy-L-leucyl-L-leucyl-L-leucinal (ZLLLH; 10 μM; Peptide Institute, Inc., Osaka, Japan), then subsequently treated with IL-1β (2.5 ng/mL) for 3 or 48 h.

Western blotting analysis

Following each treatment, the cells were lysed by adding 1× sodium dodecyl sulfate (SDS) sample buffer (0.05 M Tris–HCl, 2% w/v SDS, 6% β-mercaptoethanol and 10% glycerol). The collected lysates were sonicated for 10–15 s on ice and then centrifuged at 20,000 *g* for 15 min at 4°C. Protein amounts were determined using a protein assay kit (Bio-Rad Laboratories Inc., Hercules, CA, USA). Each protein sample (25 μg per lane) was run on a 10% SDS–polyacrylamide gel for electrophoresis at 40 mA. The separated proteins were then electroblotted onto a polyvinylidene difluoride transfer membrane using a semidry blotter. The membranes were washed once with 10 mM of Tris–HCl containing 150 mM of NaCl and 0.1% Tween 20 (TBS-T), and blocked for 1 h in TBS-T containing 5% (w/v) skim milk. After washing, polyclonal antibodies against ERK1/2, phosphorylated ERK1/2, JNK1/2, phosphorylated JNK1/2, p38 MAPK, phosphorylated p38 MAPK, IKKα,

IKKβ, IKKγ, phosphorylated IKKα/β, phosphorylated IKKγ, IκB-α and phosphorylated IκB-α (Cell Signaling Technology Inc., Beverly, MA, USA) were added separately at a dilution of 1:500 or 1:1000 in TBS-T containing 5% (w/v) skim milk or 5% bovine serum albumin, and then incubation was performed for 1 or 8 h at 4°C. Immunoreactive bands were visualized using a western blot detection system (Cell Signaling).

Preparation of nuclear extracts

Cell nuclei were isolated, and the extracts were prepared as described previously (9). Briefly, nuclei were treated with buffer A [10 mM HEPES, 1.5 mM $MgCl_2$, 10 mM KCl and 0.5 mM dithiothreitol (DTT)] and then stirred for 60 min at 4°C in buffer B (20 mM HEPES, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.42 M NaCl, 25% glycerol and 0.5 mM DTT). Nuclear extracts were obtained by centrifugation for 60 min at 25,000*g* and desalted by passage through a Sephadex G-25 column (Amersham Pharmacia Biotech, Amersham, UK) equilibrated with buffer C (20 mM HEPES, 0.1 M KCl, 0.2 mM EDTA and 0.5 mM DTT). Protein concentrations were measured using a protein assay kit.

Gel mobility shift assay

A gel mobility shift assay was performed as described previously (10). Briefly, binding reactions were performed with 5 μg of sample protein in a mixture of 2 mM of Tris, 8 mM of NaCl, 0.2 mM of EDTA, 0.8% (v/v) glycerol, 0.2 mM of DTT and 20,000 c.p.m. of ^{32}P-labeled AP-1 or NF-κB oligonucleotide (Oncogene Science, Cambridge, MA, USA) in a final volume of 20 μL for 20 min on ice. A double-stranded oligonucleotide containing a tandem repeat of the consensus sequence for the binding site for AP-1 (-TGACTCA-) and that for NF-κB (-GGGGACTTTCC-) was end-labeled using a T4 polynucleotide kinase-[γ-^{32}P]ATP method. Each unlabeled doubled-stranded oligonucleotide was utilized as a competitor. The DNA–protein complexes were electrophoresed on native 5% polyacrylamide gels in 0.25× Tris-Borate-EDTA buffer (22 mM Tris, 22 mM boric acid and 0.6 mM EDTA). Gels were exposed to X-ray film at −80°C.

Quantification of IL-6, IL-8, PGE_2 and MMP-1

The amounts of IL-6, IL-8, PGE_2 and MMP-1 secreted into culture supernatants were separately measured using ELISA kits for human IL-6 (Techne Co., Minneapolis, MN, USA) and human IL-8 (Techne) or enzyme immunoassay (EIA) kits for human PGE_2 (Amersham) and human MMP-1 (The Binding Site, Birmingham, UK).

Measurement of DNA content

Following each treatment, the cell layers were washed four times with Dulbecco's modified Eagle's medium. The DNA content in each cell layer was determined using the method of Labarca and Paigen (11), with calf thymus DNA as the standard.

Statistical analysis

All experiments were performed three times, with each experiment for each cell population of HPL cells conducted in triplicate, after which the means and standard deviations were calculated. The statistical significance of differences among each group was examined by one-way ANOVA and a *post hoc t* test paired. The *post hoc t* test was performed when the ANOVA indicated significance at a level of $p < 0.05$.

Results

Interleukin-1β activates MAPKs, AP-1, IKKs, IκB-α and NF-κB

Phosphorylation of MAPKs, IKKα/β/γ and IκB-α, (Fig. 1) as well as the DNA binding activity of AP-1 and NF-κB (Fig. 2), were determined using western blotting and gel mobility shift assays, respectively, in HPL-1 cells treated with 2.5 ng/mL of IL-1β. Phosphorylated forms of MAPKs (ERK1/2, p38 MAPK and JNK1/2) were stimulated to show expression between 10 and 30 min after beginning treatment (Fig. 1A). Furthermore, the specific binding of AP-1 to its consensus sequence was stimulated at 1–3 h after treatment (Fig. 2A). Interleukin-1β-stimulated phosphorylation of IKKα/β/γ was first detected 10 min after beginning treatment, and phosphorylation and degradation of IκB-α induced by treatment with IL-1β were also observed at 10 min (Fig. 1B). In addition, IL-1β stimulated the DNA binding activity of NF-κB at 1–3 h after treatment (Fig. 2A). The DNA binding activity of AP-1, as well as NF-κB, induced by IL-1β was apparently specific, as it was completely abrogated by pre-incubation with a

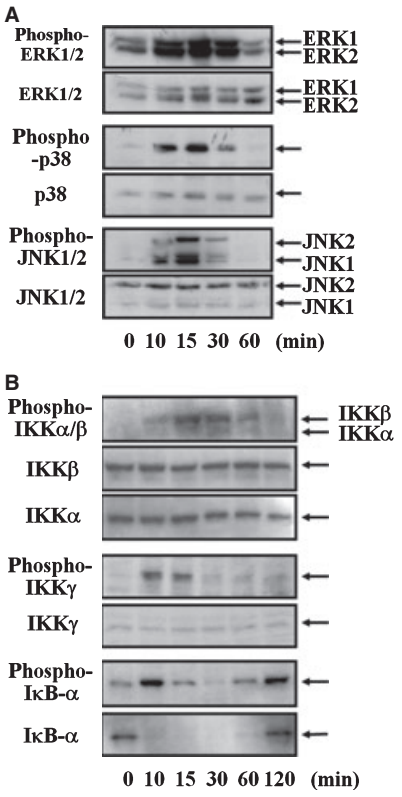

Fig. 1. Effects of IL-1β on activation of MAPK/AP-1 and IKKα/β/IκB-α/NF-κB. After HPL-1 cells were treated with IL-1β for the indicated periods, phosphorylation of MAPKs (ERK1/2, p38 MAPK and JNK1/2; A) and of IKKα/β/γ and IκB-α (B) were determined. Each photograph is representative of the results of one of three separate experiments. Similar results were obtained with HPL-2 and -3 cells (data not shown).

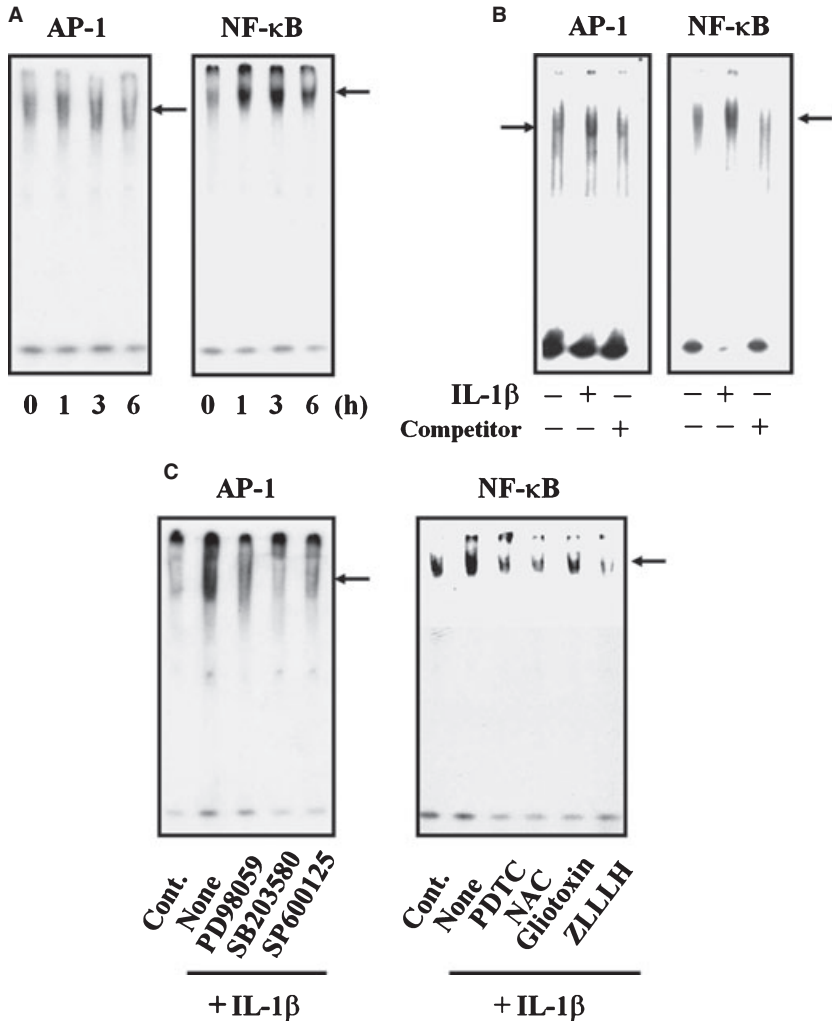

Fig. 2. Roles of inhibitors of MAPKs and NF-κB in IL-1β-stimulated DNA binding of AP-1 and NF-κB. (A) The HPL-1 cells were treated with IL-1β for 1, 3 or 6 h. (B) Nuclear protein extracted from HPL-1 cells treated with IL-1β for 1 h was incubated for 15 min with unlabelled oligonucleotide or nothing before addition of the radiolabelled probe. (C) The HPL-1 cells were pretreated for 30 min with each MAPK inhibitor, each inhibitor of NF-κB activation or nothing, then subsequently treated with IL-1β for 3 h. Following each treatment, the DNA binding activities of AP-1 and NF-κB were determined. Each photograph is representative of the results of one of three separate experiments. Similar results were obtained with HPL-2 and -3 cells (data not shown).

10-fold molar excess of each unlabelled specific homologous oligonucleotide (Fig. 2B). The activation of MAPKs/AP-1 and IKKs/IκB-α/NF-κB induced by IL-1β was also seen in HPL-2 and -3 cells (data not shown).

Effects of inhibitors of MAPK and NF-κB on IL-1β-stimulated DNA binding activities of AP-1 and NF-κB

We investigated the role of each MAPK with regard to the DNA binding of AP-1 induced by IL-1β in human periodontal ligament fibroblasts using specific MAPK inhibitors. When HPL-1 cells were pretreated for 30 min with the MAPK inhibitors, which were PD098059 (ERK1/2 inhibitor; 1 μM), SB203580 (p38 MAPK inhibitor; 1 μM) and SP600125 (JNK1/2 inhibitor; 10 μM), then subsequently treated with IL-1β (2.5 ng/mL) for 1 h, the IL-1β-stimulated DNA binding level of AP-1 was partly inhibited by each inhibitor to various extents (Fig. 2C). However, none of the MAPK inhibitors had an effect on IL-1β-induced NF-κB binding (data not shown). The effects of these inhibitors were also observed in HPL-2 and -3 cells (data not shown).

Next, to determine the effects of NF-κB inhibitors on IL-1β-stimulated NF-κB DNA binding in HPL cells, HPL-1 cells were pretreated for 30 min with each inhibitor of NF-κB activation, which were the antioxidants, PDTC (2 μM) and NAC (0.1 μM), the IκB-α phosphorylation and/or degradation inhibitor gliotoxin (1 μM), and the proteasome inhibitor, ZLLLH (10 μM), then subsequently with IL-1β (2.5 ng/mL) for 3 h. Each of the NF-κB inhibitors partly inhibited the IL-1β-stimulated DNA binding level of NF-κB to various extents (Fig. 2C). However, none of the inhibitors tested had an effect on IL-1β-enhanced AP-1 binding (data not shown). The effects of these inhibitors were also observed in HPL-2 and -3 cells (data not shown).

Inhibitors of MAPK and NF-κB suppress IL-1β-stimulated IL-6, IL-8, PGE_2 and MMP-1 production to various extents

Finally, we examined the involvement of both MAPK and NF-κB in IL-1β-stimulated IL-6, IL-8, PGE_2 and MMP-1 production by HPL cells. Treatment with IL-1β (2.5 ng/mL) for 48 h strongly stimulated IL-6, IL-8, PGE_2 and MMP-1 production in the three populations of HPL cells (Fig. 3).

Next, the three HPL cell populations were pretreated separately with each of the MAPK inhibitors, which were U0126 (1 μM), PD098059 (1 μM), SB203580 (1 μM) and SP600125 (10 μM), for 1 h, then further treated with IL-1β (2.5 ng/mL) for 48 h. As shown in Fig. 3A, IL-1β-stimulated IL-6 and IL-8 production was partly, but significantly, inhibited by U0126 (IL-6, 51% inhibition; IL-8, 57% inhibition), PD098059 (IL-6, 43% inhibition;IL-8, 46% inhibition) and SP600125 (IL-6, 40% inhibition; IL-8, 46% inhibition). The inhibitory effect of SB203580 on the production of these IL-1β-stimulated cytokines was greater than that of the other MAPK inhibitors (IL-6, 71% inhibition; IL-8, 72% inhibition). All of the MAPK

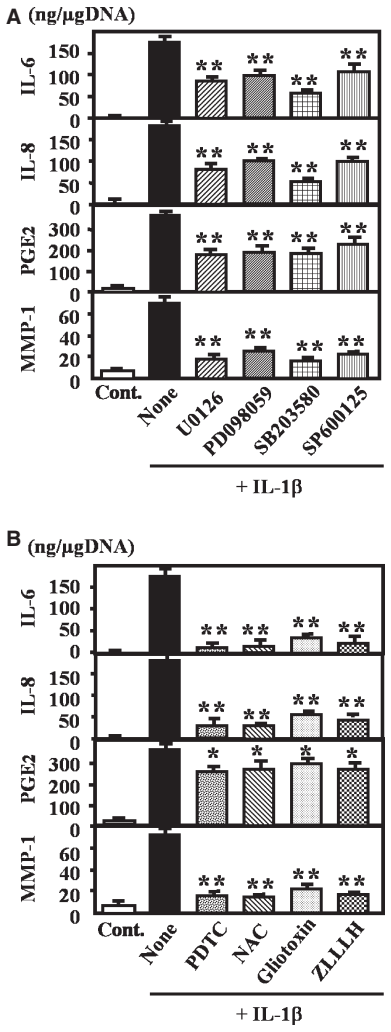

Fig. 3. Inhibition of IL-1β-induced IL-6, IL-8, PGE_2 and MMP-1 production by AP-1 and NF-κB inhibitors. Each of the three cell populations of HPL cells was pretreated for 1 h with each indicated MAPK inhibitor (A) or NF-κB inhibitor (B), then subsequently treated with IL-1β for 48 h. Following each treatment, the amounts of IL-6, IL-8, PGE_2 and MMP-1 secreted into culture supernatants were measured. Values (in nanograms per microgram DNA) are shown as the means ± SD of three separate experiments, each conducted in triplicate, for each of the three cell populations of HPL cells. Differences from the value for cells treated with IL-1β alone were considered significant at $*p < 0.05$ and $**p < 0.01$.

inhibitors also partly, but significantly, suppressed IL-1-stimulated PGE_2 production (percentage inhibition: U0126, 54%; PD098059, 49%; SB203580, 49%; SP600125, 45%; Fig. 3A). Furthermore, they strongly inhibited IL-1β-stimulated MMP-1 synthesis (percentage inhibition: U0126, 75%; PD098059, 67%; SB203580, 76%; SP600125, 72%; Fig. 3A).

When the three HPL cell populations were pretreated separately with each of the NF-κB inhibitors, which were PDTC (2 μM), NAC (0.1 μM), gliotoxin (1 μM) and ZLLLH (10 μM), for 1 h, then subsequently treated with IL-1β (2.5 ng/mL) for 48 h, each inhibited IL-1β-stimulated IL-6, IL-8 and MMP-1 production (percentage inhibition for IL-6, IL-8 and MMP-1, respectively: PDTC, 91, 84 and 79%; NAC, 90, 84 and 83%; gliotoxin, 80, 71 and 71%; ZLLLH, 86, 74 and 76%; Fig. 3B). The inhibitors also weakly, but significantly, reduced IL-1β-stimulated PGE_2 production (percentage inhibition: PDTC, 32%; NAC, 29%; gliotoxin, 22%; ZLLLH, 28%; Fig. 3B).

Discussion

The IL-1β and TNF-α signaling cascades include three distinct types of MAPKs: ERK, p38 MAPK and JNK (12,13). In the present study, we demonstrated that IL-1β rapidly stimulates the phosphorylation of each of these in HPL cells. Furthermore, it was previously shown that IL-1 and TNF-α enhance AP-1 DNA binding activity in gingival fibroblasts and synovial fibroblasts (14–16) and, after binding to AP-1 binding sites in promoters, AP-1 activates the transcription of various target genes in response to cytokines (17). We also found that IL-1β treatment of HPL cells resulted in the enhancement of AP-1 DNA binding. The three types of MAPKs regulate the activation of AP-1. In the present study, IL-1β-stimulated AP-1 DNA binding activity was inhibited by pretreatment with each inhibitor for ERK1/2, p38 and JNK1/2 to different extents. These results indicate that when the three MAPKs are activated simultaneously by IL-1β, they mediate the subsequent DNA binding of AP-1 in HPL cells, though each pathway may play a distinct role in the induction of IL-1β activity.

In the present study, IL-1β rapidly stimulated the phosphorylation of IKKα/β/γ, phosphorylation and degradation of IκB-α and subsequent DNA binding of NF-κB in HPL cells. In addition, pretreatment with each NF-κB inhibitor partly inhibited the subsequent IL-1β-enhanced DNA binding level of NF-κB in the cells. These findings confirm the involvement of IKKs/IκB-α/NF-κB in the IL-1 signaling cascade in HPL cells. Although IL-1β engages both the MAPK/AP-1 and IKK/IκB/NF-κB cascades, these two pathways may run on the same axis or in parallel. In the present study, none of the MAPK inhibitors had an effect on IL-1β-induced NF-κB binding, while none of the NF-κB inhibitors had an effect on IL-1β-enhanced AP-1 binding (data not shown). These results suggest that the two IL-1β-activated pathways are primarily dissociated, and support previous reports that have demonstrated divergence of the NF-κB and p38 MAPK cascades in various cell types treated with TNF-α (15,18–20).

As shown in previous reports (4–7), the present study demonstrated that IL-1β strongly stimulates IL-6, IL-8, PGE_2 and MMP-1 production in HPL cells. In the present study, all of the MAPK inhibitors partly, but significantly, suppressed IL-1β-stimulated IL-6 and IL-8 production. In addition, the inhibitory effect of the p38 MAPK inhibitor was greater than that of the ERK and JNK inhibitors. The dominant role of p38 MAPK in the synthesis of proinflammatory cytokines has been shown, because activation of p38 MAPK by IL-1β contributed to IL-6 expression through mRNA stability in rheumatoid synovial fibroblasts and osteoblasts (21,22). Likewise, SB203580 significantly inhibited IL-1β-stimulated IL-6 and IL-8 production at the protein level, but not at the mRNA level, in gingival fibroblasts, dermal fibroblasts and umbilical vein endothelial cells, as well as rheumatoid synovial fibroblasts (23,24).

We previously demonstrated that IL-1β-induced PGE_2 generation in HPL cells was primarily dependent on *de novo* induction of cyclooxygenase-2 (COX-2) and cytosolic phospholi-

pase A2 (cPLA2) (6). A number of previous studies have also suggested that PGE_2 production and upstream cPLA2/COX-2 expression induced by IL-1β in various cell types are mediated through the activation of ERK, p38 MAPK and/or JNK signaling pathways (25–31). Furthermore, p38 MAPK (32,33) or ERK (27) acts to stabilize COX-2 mRNA induced by IL-1β. Another study demonstrated that a p38 inhibitor strongly suppressed IL-1-induced PGE_2 production in fibroblasts and endothelial cells (23). We also found that all three MAPK inhibitors partly, but significantly, reduced PGE_2 production by IL-1β-treated HPL cells, suggesting that activation of each MAPK pathway, at least in part, plays a role in the signaling cascades that mediate the up-regulation of PGE_2 production in cells exposed to IL-1β.

A previous study revealed the important role of p38 MAPK in *MMP-1* gene and protein expression by gingival fibroblasts (34). Another study that used synovial fibroblasts demonstrated that IL-1-induced *MMP-1* gene expression involved ERK as well as p38 MAPK, and the ERK pathway primarily regulated gene transduction (35). However, in a separate study of synovial fibroblasts, a JNK inhibitor completely blocked IL-1-induced *MMP-1* mRNA expression, while p38 MAPK inhibition had no effect, and ERK inhibition had only a modest effect (16). In the present study, each inhibitor of the three types of MAPK remarkably inhibited IL-1β-stimulated MMP-1 synthesis at nearly the same level.

The roles of NF-κB in IL-6, IL-8, PGE_2 and MMP-1 generation have been widely investigated. Nuclear factor-κB inhibitors reduced IL-6 and IL-8 production by rheumatoid synovial fibroblasts (36,37). Furthermore, spontaneous and IL-1-stimulated expressions of IL-6 and IL-8 by rheumatoid synovial fibroblasts and dermal fibroblasts were significantly suppressed by employing an IκB-α-expressing adenovirus, and mouse embryonic fibroblasts isolated from NF-κB knockout mice failed to express the *IL-6* gene in response to IL-1 (38). These observations indicate that NF-κB is a major contributor to production of these cytokines by fibroblasts. Previous evidence also suggested a potent role of NF-κB in the regulation of IL-1-induced MMP-1 production in several types of fibroblasts (39–41). Our data support those previous findings, because all of the NF-κB inhibitors tested strongly suppressed the production of IL-6, IL-8 and MMP-1 by HPL cells. In contrast, we found that the inhibitory effects of the NF-κB inhibitors on IL-1β-stimulated PGE_2 generation were relatively weak.

In summary, we comprehensively investigated the involvement of MAPK/AP-1 and NF-κB in IL-1β-induced IL-6, IL-8, PGE_2 and MMP-1 production by HPL cells. As shown in Fig. 4, the three types of MAPK (ERK1/2, p38 MAPK and JNK1/2), all of which were activated simultaneously by IL-1β, mediated the subsequent DNA binding of AP-1 to various degrees, while IKKα/β/γ/IκB-α/NF-κB was also involved in the IL-1 signaling cascade. Interleukin-1β stimulated HPL cells to produce IL-6, IL-8, PGE_2 and MMP-1 via activation of the three MAPKs and NF-κB. However, those pathways may play a distinct role in their production induced by IL-1β. Taken together, our findings strongly suggest that the MAPKs/AP-1 and IKKα/β/IκB-α/NF-κB cascades mediate the IL-1β-stimulated synthesis of IL-6, IL-8, PGE_2 and MMP-1 in HPL cells. In our previous study, similar results were obtained in human gingival fibroblasts, which are a major constituent of periodontium (42). In addition, inhibition of p38 MAPK or NF-κB was especially effective for suppressing IL-1β activity. These signal transduction pathways are also activated by other proinflammatory factors, including TNF-α and lipopolysaccharide. Therefore, local administration of p38 MAPK inhibitors and inhibitors of NF-κB activation, such as proteasome inhibitors and IKK inhibitors, may be therapeutically and clinically useful for treatment of destructive periodontitis.

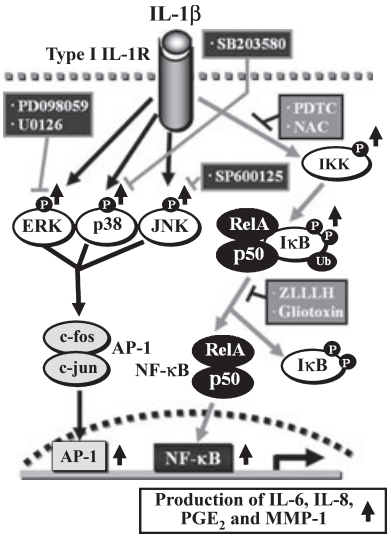

Fig. 4. The MAPKs/AP-1 and IKKα/β/IκB-α/NF-κB cascades mediate IL-1β-stimulated synthesis of IL-6, IL-8, PGE_2 and MMP-1 in HPL cells.

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