Interleukin-6 production in human fibroblasts derived from periodontal tissues is differentially regulated by cytokines and a glucocorticoid


Interleukin-6 (IL-6) is thought to be a major mediator of the host's defense against infection, and it regulates immune responses in inflamed tissue. In this study, we investigated the regulation of IL-6 production in human gingival fibroblasts (HGF) and human periodontal ligament fibroblasts (HPLF). Pro-inflammatory cytokines including interleukin (IL)-1α, IL-1β and tumor necrosis factor (TNF)-α stimulated IL-6 production in HGF and HPLF in a time- and dose-dependent manner. This IL-1α, IL-1β, or TNF-α-induced IL-6 production was enhanced, but the cAMP accumulation they induced was inhibited by the addition of indomethacin. This result suggests that endogenous prostaglandin E2 (PGE2) partially inhibits IL-1α or TNF-α-induced IL-6 production, and that the enhancement of IL-6 production by IL-1α or TNF-α may not be caused through endogenous PGE2-induced cAMP-dependent pathway. Dexamethasone (DEX), a glucocorticoid which is an inhibitor of nuclear factor kappa B (NF-κB) activation, markedly inhibited IL-1α or TNF-α-induced IL-6 production; so this production may be partially mediated through NF-κB. IL-1α or TNF-α enhanced IL-6 production synergistically. IL-6 production in HGF or HPLF stimulated with IL-1β was augmented by the addition of interferon (IFN)-γ, but was slightly suppressed by the addition of IL-4. Endogenous IL-6 enhanced IL-1β or TNF-α-induced IL-6 production in the presence of IL-6 soluble receptor (IL-6sR). Accordingly, in inflamed periodontal tissues, gingival fibroblasts and periodontal ligament fibroblasts stimulated with pro-inflammatory cytokines such as IL-1α or TNF-α may produce IL-6, and this production can be differentially modulated by endogenous PGE2, IL-6sR, T cell-derived cytokines such as IFN-γ or IL-4, and glucocorticoids.

Recently the involvement of cytokines, produced by immune and non-immune cells in the initiation and progression of periodontal disease, has received considerable attention (1–3). Interleukin-6 (IL-6) is a multifunctional cytokine produced by both lymphoid and non-lymphoid cells (4, 5), and is thought to have a major role in the regulation of immune responses (6). IL-6 has a wide variety of biological activities on immunocompetent cells. This cytokine induces differentiation and antibody secretion in B cells, activation and differentiation in T cells, and differentiation in macrophages (6–8). IL-6 also stimulates osteoclastic bone resorption via an effect on osteoclast formation (9–11).

The involvement of IL-6 in the regulation of local immune and inflammatory responses in periodontal disease has been recognized because of similarities between its known biological effects and the
manifestations of the disease, as well as its presence in lesions. Several clinical observations demonstrated that IL-6-producing cells were identified in inflamed gingiva (12, 14, 15), and that IL-6 was detected in the gingival crevicular fluid of periodontitis patients (13).

The functions of fibroblasts are regulated by various cytokines produced by the cells of the immune system, but fibroblasts also produce various cytokines themselves (16). This indicates that fibroblasts can also be included in the cytokine network, and may play an important role in local inflammatory and immune responses. Actually, fibroblasts produce IL-6. Some evidence indicates that various stimuli including cytokines and lipopolysaccharides (LPS) enhance IL-6 production in fibroblasts (17–23). Furthermore, in inflamed gingiva, IL-6 mRNA is expressed not only in macrophages, lymphocytes and endothelial cells, but also in fibroblasts (24). However, there are few reports regarding the regulation of IL-6 production in human gingival fibroblasts (HGF) or human periodontal ligament fibroblasts (HPLF) (12, 25, 35).

In this study, we investigated the regulation of IL-6 production in fibroblasts in inflamed periodontal tissue by analyzing the regulatory effects of cytokines, endogenous prostaglandin E2 (PGE2), IL-6 soluble receptors (IL-6sR) and a glucocorticoid, alone or in combination, on IL-6 production in HGF or HPLF in vitro.

Material and methods

Reagents

Recombinant human interleukin-1 (rhIL-1)α (specific activity: 1 x 10^8 unit (U)/mg), rhIL-1β (specific activity; 5 x 10^8 U/mg), rh tumor necrosis factor (TNF)-α (specific activity; 2 x 10^7 U/mg), rh interleukin-4 (IL-4) (specific activity; 1 x 10^7 U/mg), and rh interferon (IFN) -γ (specific activity; 2 x 10^7 U/mg) were purchased from Genzyme Co. (Boston, MA). RhIL-6sR was purchased from R&D Systems (Minneapolis, MN). Indomethacin (INDO) and dexamethasone (DEX) was obtained from Sigma Chemical Co. (St Louis, MO).

Cell culture

The human periodontal ligaments attached to the middle-third of the root were collected with a sharp surgical scalpel from premolars extracted from 8 patients with clinically healthy periodontium during the course of orthodontic treatment. The attached gingiva were obtained from the same 8 patients. All subjects were duly informed of the nature and extent of the experiments, after which their informed consent was obtained according to the Helsinki Declaration. The human gingiva and periodontal ligament collected were washed 4 times with phosphate-buffered saline (PBS; Takara Shuzo Co., Siga, Japan), minced and placed into 35-mm^2 tissue culture dishes (Corning, New York, NY, USA). Then, these tissues were cultured in Dulbecco's Modified Eagle's medium (DMEM; Flow Laboratories, McLean, VA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), 100 U/ml penicillin G (Banyu pharmaceutical Co., Tokyo, Japan) and 100 μg/ml streptomycin (Meiji Kaisha Ltd, Tokyo, Japan). The cultures were maintained at 37°C in 95% air and 5% CO2 until confluent cell monolayers were formed. After subculturing 3 times homogeneous, slim, spindle-shaped cells were obtained. These human gingival fibroblasts (HGF) and human periodontal ligament fibroblasts (HPLF) were designated HGF-1 to 8 and HPLF-1 to 8, respectively. These cells were used at passages 4 and 5 in all the experiments. The HPLF used in this study had phenotypes of osteoblasts, namely, intense alkaline phosphatase activity, PTH-dependent cAMP production and bone gla protein synthesis in response to 1, 25(OH)2D3 (data not shown).

Measurement of IL-6

HGF or HPLF was seeded in 24-well flat-bottomed tissue culture plates (Corning) at a concentration of 1 x 10^4 cells/well and cultured in 800 μl of DMEM containing 10% FBS until confluent. Then the cell layers of HGF or HPLF were washed 3 times with DMEM and subsequently treated with rhIL-1α (0.005–5 ng/ml), rhIL-1β (0.005–5 ng/ml), rhTNF-α (0.1–100 ng/ml), rhIL-6sR (100 ng/ml), rhIFN-γ (1–10^3 U/ml), or rhIL-4 (0.1–10 ng/ml), alone or in combination, in 800 μl of DMEM containing 2% FBS for 24, 48 or 72 h. Some confluent HGF or HPLF cultures were treated with 0.5 ng/ml of rhIL-1 (α or β) or 10 ng/ml of rhTNF-α in the presence or absence of DEX (10^-12 to 10^-6 mol (M)) or 10^-6 M of INDO for 48 h. At the end of each treatment period, the culture supernatant was collected. The amount of IL-6 in the supernatant was measured with a human IL-6 enzyme immunoassay (EIA) kit (Genzyme). The cell layers were washed 4 times with DMEM and their DNA contents were measured. The DNA content was measured by the method of Labarca & Paigen (27) using calf thymus DNA as the standard.

Measurement of cAMP accumulation and PGE2 production

HGF or HPLF (1 x 10^4 cells/well) were plated in 24-well flat-bottomed tissue culture plates, and were
cultured for 7 d, until confluent. Then the cell layers of HGF or HPLF were washed 3 times with DMEM and treated subsequently with or without 0.5 ng/ml of rhIL-1 (α or β) or 10 ng/ml of rhTNF-α in the presence or absence of 10⁻⁶ M of INDO for 48 h. At the end of this treatment period the culture supernatant was collected and used for PGE₂ measurements. The amount of PGE₂ was determined with a human PGE₂ EIA kit (Amersham Co., Buckinghamshire, UK). The cell layers were washed 4 times with DMEM containing 0.5% bovine serum albumin (BSA) (Sigma). After that, cAMP in the cell layer was extracted with 500 μl of acidic ethanol at 4°C for 2 h, and acidic ethanol was evaporated by boiling. The remaining pellet was dissolved in 200 μl of 3 mM HCl. The amount of cAMP in this solution was determined with a radioimmunoassay kit for cAMP (Yamasa Shoyu Co., Chiba, Japan).

Statistical analysis
Each assay was performed in triplicate cultures and the means and standard deviations (s.d.) were calculated. All experiments were repeated 3 times and representative examples from triplicate experiments are shown in the Results section. Statistical evaluation was performed by Student’s t-test for paired data and considered significant if the p values were less than 0.05.

Results
Induction of IL-6 production in HGF or HPLF by treatment with pro-inflammatory cytokines
Small amounts of IL-6 were detected in the supernatants of untreated HGF-1 and HPLF-1 cultures throughout each treatment period. However, 0.5 ng/ml of rhIL-1 (α or β) or 10 ng/ml of rhTNF-α stimulated IL-6 production. The amounts of IL-6 induced by these cytokines increased time-dependently until 24 h in HGF-1 or until 48 h in HPLF-1, respectively (Fig. 1). RhIL-1α (0.005 ~ 0.5 ng/ml), rhIL-1β (0.005 ~ 0.5 ng/ml) or rhTNF-α (0.1 ~ 10 ng/ml) all stimulated IL-6 production in HGF-1 and HPLF-1 (Fig. 2). RhIL-1α or β stimulated this production more strongly than rhTNF-α in HGF-1 and HPLF-1 (Figs 1, 2). HGF-1 produced larger amounts of IL-6 than HPLF-1 when treated with these cytokines (Figs 1, 2). These results were also obtained in HGF-2, 3 and, and HPLF-2, 3 and 4 (Table 1).

Effect of a combination of rhIL-1 and rhTNF-α on IL-6 production
RhTNF-α (1 ~ 10 ng/ml) dose-dependently enhanced IL-6 production in HGF-4 and HPLF-4 treated with 0.5 ng/ml of rhIL-1 (α or β) for 48 h (Fig. 3). As shown in Table 2, this synergistic enhancement by rhIL-1 (α or β) and rhTNF-α on IL-6 production in HGF and HPLF was also observed in HGF-2 and 3, and HPLF-2 and 3.

Effect of endogenous PGE₂ on rhIL-1- or rhTNF-α-induced IL-6 production and cAMP accumulation
We found that 0.5 ng/ml of rhIL-1α, 0.5ng/ml of rhIL-1β and 10 ng/ml of rhTNF-α all stimulated PGE₂ production in HGF-7 and HPLF-7, and HPLF-7 secreted larger amounts of PGE₂ than HGF-7 when stimulated with these cytokines. Furthermore, PGE₂ production was inhibited to almost control levels by the addition of INDO (10⁻⁶ M) (data not shown). INDO (10⁻⁶ M) had no effect on IL-6 production and cAMP accumulation in HGF-7 and HPLF-7 (data not shown). However, IL-6 production in HGF-7 and HPLF-7, which were treated with 0.5 ng/ml of rhIL-1 (α and β) or 10 ng/ml of rhTNF-α for 48 h, was significantly enhanced by the addition of INDO (10⁻⁶ M) (Fig. 4). These cytokines also stimulated cAMP accumulation in HGF-7 and HPLF-7, and the level of accumulation in HPLF-7 was much higher than that in HGF-7 (Fig. 4). INDO (10⁻⁶ M) caused a significant decrease in these cytokine-induced cAMP accumulations in HGF-7 and HPLF-7, and inhibition in HPLF-7 was stronger than in HGF-7 (Fig. 4). These results were also obtained in HGF-6 and 8, and HPLF-6 and 8 (data not shown).

Inhibitory effect of DEX on rhIL-1-induced IL-6 production
DEX (10⁻¹² ~ 10⁻⁶ M) dose-dependently reduced IL-6 production in HGF-4 and HPLF-5 treated with 0.5 ng/ml of rhIL-1 (α or β), or 10 ng/ml of rhTNF-α for 48 h (Fig. 5). Similar results were also obtained in HGF-6 and 7 and HPLF-6 and 7 (data not shown).

Effect of endogenous IL-6 on rhIL-1-induced IL-6 production
100 ng/ml of rhIL-6sR could not induce IL-6 production in HGF-8 and HPLF-8, but rhIL-1 (α or β)-induced IL-6 production was significantly enhanced by the addition of rhIL-6sR (100 ng/ml) (Fig. 6). Similar results were also obtained in HGF-6 and 7, and HPLF-6 and 7 (data not shown).

Effect of rhIL-4 or rhIFN-γ on rhIL-1/β-induced IL-6 production
RhIL-4 (0.1 ~ 10 ng/ml) had no effect on IL-6 production in HGF-6 and HPLF-6 (data not shown).
Fig. 1. Time course of IL-6 production in HGF-1 and HPLF-1 treated with rhIL-1 (α or β) or rhTNF-α. HGF-1 and HPLF-1 at confluence were treated with medium (○), 0.5 ng/ml of rhIL-1α (●), 0.5 ng/ml of rhIL-1β (▲) or 10 ng/ml of rhTNF-α (■) for 24, 48 or 72 h. The amounts of IL-6 in supernatant were determined by EIA. This experiment was repeated 3 times, and representative data from one of these is shown. The values shown are the means ± s.d. of triplicate cultures.

Fig. 2. Dose-dependent changes of IL-6 production in HGF-1 and HPLF-1 treated with rhIL-1 (α or β) or rhTNF-α. HGF-1 and HPLF-1 at confluence were treated with medium (■), rhIL-1α (●; 0.005~5 ng/ml), rhIL-1β (▲; 0.005~5 ng/ml), or TNF-α (□; 0.1~100 ng/ml) for 48 h. This experiment was repeated 3 times, and representative data from one of these is shown. The values shown are the means ± s.d. of triplicate cultures. n.d.: not detected.

shown). However, rhIL-4 slightly inhibited the IL-6 production induced by 0.5 ng/ml of rhIL-1β at concentrations over 0.1 ng/ml in HGF-6 and over 1 ng/ml in HPLF-6. Simultaneous treatment with 0.5 ng/ml of rhIL-1β and 1 ng/ml of rhIL-4 for 48 h inhibited IL-6 production to 82% of the level obtained with rhIL-1β treatment alone (p<0.01) in HGF-6, or to 79% of the level obtained with
Regulation of IL-6 production in fibroblasts

Table 1. IL-6 production in HGF and HPLF stimulated with several cytokines for 48 h

<table>
<thead>
<tr>
<th>Cells</th>
<th>Control</th>
<th>rhIL-1α (0.5 ng/ml)</th>
<th>rhIL-1β (0.5 ng/ml)</th>
<th>rhTNF-α (10 ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGF-</td>
<td>1</td>
<td>1.30 ± 0.06</td>
<td>601.72 ± 65.21</td>
<td>556.59 ± 60.42</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.16 ± 0.21</td>
<td>569.76 ± 34.16</td>
<td>534.64 ± 91.45</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.92 ± 0.29</td>
<td>697.49 ± 54.81</td>
<td>761.60 ± 70.29</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.58 ± 0.16</td>
<td>400.00 ± 28.36</td>
<td>553.30 ± 35.50</td>
</tr>
<tr>
<td>HPLF-</td>
<td>1</td>
<td>0.3 ± 0.06</td>
<td>140.50 ± 4.10</td>
<td>133.66 ± 17.78</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.16 ± 0.21</td>
<td>157.31 ± 10.87</td>
<td>238.66 ± 21.41</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.15 ± 0.03</td>
<td>200.40 ± 4.10</td>
<td>209.46 ± 18.09</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.21 ± 0.05</td>
<td>207.00 ± 18.75</td>
<td>220.40 ± 18.46</td>
</tr>
</tbody>
</table>

a Values are representative of the means ± s.d. of triplicate cultures.
b Not detected.

Fig. 3. Synergistic effect of rhIL-1 (α or β) and rhTNF-α on IL-6 production in HGF-4 and HPLF-4. HGF-4 (■) and HPLF-4 (■) at confluence were treated with 0.5 ng/ml of rhIL-1 (α or β) or rhTNF-α (1 or 10 ng/ml), alone or in combination, for 48 h. This experiment was repeated 3 times, and representative data from one of these is shown. The values shown are the means ± s.d. of triplicate cultures. Differences from rhIL-1α alone or rhIL-1β alone were significant at p < 0.01 (***). Table 2. Synergistic effect of rhIL-1 (α) or β) and rhTNF-α on IL-6 production in HGF and HPLF

<table>
<thead>
<tr>
<th>Cells</th>
<th>rhIL-1α/rhTNF-α (0.5 ng/ml)</th>
<th>rhIL-1β/rhTNF-α (0.5 ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGF-</td>
<td>1.47</td>
<td>1.89</td>
</tr>
<tr>
<td></td>
<td>1.85</td>
<td>1.65</td>
</tr>
<tr>
<td></td>
<td>2.68</td>
<td>2.09</td>
</tr>
<tr>
<td>HPLF-</td>
<td>1.43</td>
<td>2.43</td>
</tr>
<tr>
<td></td>
<td>1.38</td>
<td>1.73</td>
</tr>
<tr>
<td></td>
<td>1.70</td>
<td>1.69</td>
</tr>
</tbody>
</table>

a HGF or HPLF at confluence were treated with rhIL-1α or rhIL-1β alone, or in combination with rhTNF-α for 48 h.
b The ratio of the mean amount of IL-6 produced in triplicate cultures, for rhIL-1α (α) or β) + rhTNF-α vs. rhIL-1α (α or β) alone.

rhIL-1β treatment alone (p < 0.05) in HPLF-6 (Fig. 7). Similar results were also obtained in HGF-4 and 7, and HPLF-5 and 7 (Table 3). Conversely, rhIFN-γ (10 ~ 10³ U/ml) had no effect on IL-6 production in HGF-5 and HPLF-6 (data not shown). However, rhIFN-γ significantly
Fig. 4. Effect of INDO on rhIL-1 (α or β) or rhTNF-α-induced IL-6 production and cAMP accumulation in HGF-7 and HPLF-7. HGF-7 (■) and HPLF-7 (□) at confluence were treated with 0.5 ng/ml of rhIL-1 (α or β) or 10 ng/ml of TNF-α, alone or in combination with 10^{-6} M of INDO, for 48 h. The amounts of IL-6 in supernatant were determined by EIA. The cAMP accumulation in cell layer was assessed as described in Material and methods. This experiment was repeated 3 times, and representative data from one of these is shown. The values shown are the means ± s.d. of triplicate cultures. Differences from rhIL-1α alone, rhIL-1β alone, or rhTNF-α alone were significant at p <0.01 (**), p <0.02 (*), p <0.05 (*).

Enhanced 0.5 ng/ml of rhIL-1β-induced IL-6 production at concentrations over 10 U/ml in HGF-5 or over 10^2 U/ml in HPLF-6. Simultaneous treatment with 0.5 ng/ml of rhIL-1β and 10^3 U/ml of rhIFN-γ for 48 h synergistically enhanced IL-6 production in HGF-5 and HPLF-6, compared with rhIL-1β alone (1.41-fold of rhIL-1β alone in HGF-5; p <0.01, 1.72-fold of rhIL-1β alone in HPLF-6; p <0.01) (Fig. 7). Similar results were also obtained in HGF-4 and 6, and HPLF-8 and 9 (Table 4).

Discussion

Many infectious diseases are initiated and become established as a result of stimulation of cellular immunity. Local immune and inflammatory responses may be regulated through interactions between fibroblasts and inflammatory cells, which are mediated by cytokines. Fibroblasts also produce various cytokines (16). Fibroblasts are the major source of IL-6 (28). Donnelly et al. (29) demonstrated that synovial fibroblasts can be induced to express higher levels of IL-6 than human monocytes when stimulated with IL-1. Also in this study, HGF or HPLF were stimulated to produce IL-6 by treatment with rhIL-1α, rhIL-1β or rhTNF-α. This result supports previous reports concerning with IL-6 production in human gingival fibroblasts or human periodontal ligament fibroblasts (12, 25, 35). In this study, HGF produced larger amounts more rapidly of IL-6 than HPLF, when stimulated or not stimulated with these cytokines, whereas Shimizu et al. (25) demonstrated that IL-1β-stimulated IL-6 production in human periodontal ligament cells was much higher than that in Gin-1, a normal human gingival fibroblast cell line. However, we determined that HGF treated with rhIL-1β or rhTNF-α produced higher amounts of IL-6 than Gin-1 treated with these cytokines (unpublished data). Each pair of HGF and HPLF used in this study was freshly isolated from the same donor and was used at the same passages in each experiment. Therefore, we are convinced that human gingival fibroblasts have the ability to produce higher levels of IL-6 than human periodontal ligament fibroblasts when stimulated with IL-1 or TNF-α. This result may be due to differences in the number or affinity of IL-1 or TNF-α receptors, or the expression or stability of IL-6 mRNA in HGF and in HPLF.

IL-1 and TNF-α are well documented as inducers of IL-6 production in stromal cells. In this study, rhIL-1 (α or β) induced greater IL-6 production than TNF-α, not only in HGF, but also in HPLF. The greater efficiency of IL-1β than TNF-α as an inducer of IL-6 production has been shown in human foreskin fibroblasts (26) and human lung fibroblasts (30). Recently, we determined that rhTNF-α slightly induced IL-6 mRNA expression in Northern blot analysis, but rhIL-1β induced significantly this expression in HGF and HPLF (unpublished data). Therefore, this greater efficiency of IL-1β than TNF-α may be due to the difference of the regulation at pretranscriptional or transcriptional processes by IL-1β or TNF-α. Despite their different amino acid sequences, TNF and IL-1 share many biological activities because TNF and IL-1 stimulate similar intracellular messengers by different pathways, activating the same cascade of intracellular metabolism (31). However, different receptors mediate the action of TNF and IL-1, thus TNF and IL-1 can act independently on
target cells. Consequently, the biological effects of these 2 cytokines are often synergistic (31). Also in this study, rhIL-1 (α or β) and rhTNF-α synergistically enhanced IL-6 production in HGF and HPLF.

It is known that IL-1 and TNF-α can stimulate PGE2 production in fibroblasts in vitro (32–34). In this study, PGE2 production in HGF and HPLF was enhanced by treatment with rhIL-1β or rhTNF-α. Furthermore, rhIL-1β or rhTNF-α-induced
PGE₂ production was inhibited to almost control levels by the addition of INDO (10⁻⁶ M). We also showed that the rhIL-1 (α or β) or rhTNF-α-induced IL-6 production in HGF or HPLF was enhanced by the addition of 10⁻⁶ M of INDO. These results suggest that endogenous PGE₂ partially inhibits the IL-6 production induced by IL-1 or TNF-α in HGF or HPLF, and support a previous report in human gingival fibroblasts by Takigawa et al. (35). Furthermore, these cytokines caused significant elevation in the accumulation of cAMP by HGF or HPLF. This elevation seems to be dependent on the PGE₂ produced, in part, because it was partially inhibited by the addition of INDO (10⁻⁶ M). Accordingly, as shown in this study, HPLF may produce higher levels of cAMP in response to rhIL-1 (α or β) or rhTNF-α than HGF, since these cells can produce larger amounts of PGE₂ than HGF when stimulated with these cytokines. In fact, cAMP agonists including PGE₁, forskolin or 3-isobutyl-1-methylxanthine (IBMX) stimulate cAMP accumulation in human lung fibroblasts (30). PGE dependent-cAMP accumulation was also observed in human periodontal
Regulation of IL-6 production in fibroblasts

Table 4. Synergistic effect of rhIFN-γ and rhIL-1β on IL-6 production in HGF and HPLFa

<table>
<thead>
<tr>
<th>Cells</th>
<th>rhIFN-γ (1000 U/ml)</th>
<th>rhIL-1β (0.5 ng/ml)</th>
<th>rhIL-1β + rhIFN-γ (0.5 ng/ml) (1000 U/ml)</th>
<th>Ratiob</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGF-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.94 ± 0.12</td>
<td>600.00 ± 80.50</td>
<td>1157.50 ± 93.50***</td>
<td>1.93</td>
</tr>
<tr>
<td>5</td>
<td>1.84 ± 0.20</td>
<td>510.00 ± 48.25</td>
<td>720.00 ± 21.21***</td>
<td>1.41</td>
</tr>
<tr>
<td>6</td>
<td>1.08 ± 0.12</td>
<td>552.60 ± 30.84</td>
<td>854.80 ± 36.08***</td>
<td>1.55</td>
</tr>
<tr>
<td>HPLF-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.24 ± 0.05</td>
<td>393.70 ± 39.88</td>
<td>677.60 ± 48.12***</td>
<td>1.72</td>
</tr>
<tr>
<td>8</td>
<td>0.35 ± 0.07</td>
<td>383.32 ± 14.64</td>
<td>539.00 ± 41.00***</td>
<td>1.41</td>
</tr>
<tr>
<td>9</td>
<td>0.18 ± 0.04</td>
<td>290.04 ± 14.16</td>
<td>488.00 ± 35.28***</td>
<td>1.68</td>
</tr>
</tbody>
</table>

a HGF or HPLF at confluence were treated with rhIFN-γ or rhIL-1β, alone or in combination, for 48 h.
b Values are representative of the means ± s.d. of triplicate cultures.

*** Difference from rhIL-1β alone was significant at p < 0.01.

ligament fibroblasts (33) and bovine gingival or periodontal ligament fibroblasts (36). The roles of cAMP and protein kinase C (PKC) on mediating the IL-6 production induced by IL-1 or TNF have not been determined. Zhang et al. (37, 38) reported that rIL-1 or rTNF-induced IL-6 production is partially cAMP-mediated and PKC-independent in FS-4 human foreskin fibroblasts. In contrast, Sehgal et al. (39) demonstrated that the effect of rTNF and rIL-1 is at least partially PKC-mediated and is resistant to inhibition by H-8, which is a preferential inhibitor of cAMP and cGMP-dependent protein kinases, in FS-4 cells. Zitnik et al. (30) revealed that rIL-1-induced IL-6 production in CCL-202 human lung fibroblasts is cAMP-independent, since cAMP agonists including forskolin, dibutyryl cAMP, PGE_1 and IBMX inhibit rIL-1-induced IL-6 production. As described above, we showed that endogenous PGE_2 inhibited IL-1 (α or β) or TNF-α-induced IL-6 production, but enhanced the cAMP accumulation induced by these cytokines in HGF and HPLF. This result suggests that IL-1 or TNF-α-induced IL-6 production in HGF and HPLF may not be caused through the endogenous PGE_2-induced cAMP-dependent pathway.

In this study DEX, which is a glucocorticoid, strongly inhibited IL-6 production in rhIL-1 (α or β) or rhTNF-α-treated HGF and HPLF. The inhibitory effect of DEX on IL-6 production was also observed in LPS-treated human monocytes, and IL-1-treated human endothelial cells and human skin fibroblasts (40). This indicates that DEX is profoundly involved in the regulation of IL-6 production in various cell types. The mechanism(s) by which DEX inhibits IL-6 production is not clear; however, Waage et al. (40) demonstrated that DEX, via a receptor-mediated mechanism, inhibits IL-6 production at the transcriptional level in the murine macrophage cell line RAW 264.9. This mechanism of action is further supported by the demonstration of a glucocorticoid-responsive element located in the IL-6 gene (41). Also, it is known that DEX is a potent inhibitor of nuclear factor kappa B (NF-κB) activation (46). Consequently, our result suggests that IL-1 or TNF-α-induced IL-6 production in HGF or HPLF may be partially mediated through NF-κB.

Recently, we determined that IL-6 had no effect on IL-6 production in HGF and HPLF (unpublished data). This result suggests that this may result from the low levels of IL-6 receptors expression on HGF and HPLF. IL-6 exerts its activity by binding to a high affinity receptor complex consisting of 2 membrane glycoproteins: an 80 kDa IL-6-binding receptor protein (IL-6R) and a signal-transducing protein, gp130. When combined with the IL-6/IL-6R complex, membrane-bound gp130 is involved in the formation of a high-affinity IL-6 receptor and subsequent IL-6 signal transduction (43). The gp130 is expressed in nearly all cell types. The 80 kDa IL-6R is also expressed in monocytes, hepatocytes, activated B cells, CD4 T cells and CD8 T cells and CD34+ hematopoietic progenitor cells (44, 45), but some cell lines lack this receptor (43). We are now investigating the expression of IL-6R and gp130, proteins and transcripts in HGF and HPLF. A soluble form of IL-6R (IL-6sR) with a molecular weight of approximately 50 kDa may be released from the cell surface by proteolytic cleavage but the precise mechanism is unknown, as the IL-6sR transcript encoding a soluble form might not have been identified because of its low abundance (43). A natural soluble form of IL-6R, which is capable of binding IL-6, is present in human plasma. A complex of IL-6 and IL-6sR can be associated with gp130, and mediate IL-6 signals through gp130. Mihara et al. (46) demonstrated that IL-6sR alone cannot induce synovial fibroblast proliferation, but it augments IL-1-β-induced
proliferation, which suggests that IL-6 induced by IL-1β is very likely to be involved in synovial fibroblast proliferation in cooperation with IL-6sR. In this study, IL-6sR did not stimulate IL-6 production in HGF or HPLF. However, rhIL-1 (α or β)-induced IL-6 production was enhanced in the presence of IL-6sR. These results suggest that HGF and HPLF may lack or express low levels of IL-6R but express gp130, so endogenous IL-6 has no effect on IL-1-induced IL-6 production, but it enhances this production in the presence of IL-6sR. Consequently, IL-6 may be auto-stimulatory, in that IL-6 amplifies and perpetuates its own production in HGF and HPLF in the presence of IL-6sR.

In this study we investigated the effects of T cell-derived cytokines, such as IFN-γ and IL-4, on IL-6 production in HGF and HPLF. RhIFN-γ and rhIL-4 had no effect on IL-6 production in HGF or HPLF. However, simultaneous stimulation with rhIL-1β and rhIFN-γ enhanced the IL-6 production induced by treatment with rhIL-1β alone, whereas a combination of rhIL-1β and rhIL-4 slightly inhibited this production. These results suggest that IFN-γ and IL-4 may regulate differentially IL-1-induced IL-6 production in HGF and HPLF. The mechanisms of regulations are still poorly understood. Very recently, we showed that rhIFN-γ-treated HGF increased, but rhIL-4-treated HGF reduced, 125I-IL-1 binding and this change of binding was due to an increase or a decrease in the number of IL-1 receptors, not to changes in affinity (unpublished data), so IFN-γ and IL-4 may differentially regulate IL-1β-induced IL-6 production in HGF and HPLF via the differential regulation of IL-1 receptor expression. However, Donnelly et al. (29) demonstrated that IL-4 inhibited IL-1-induced IL-6 production and NF-κB activity in monocytes, but not in synovial fibroblasts and in normal fibroblast lines derived from other tissues. However, the inability of IL-4 to suppress IL-6 synthesis in fibroblasts was not caused by a lack of IL-4 receptors (IL-4R). In this study, the inhibitory effect of IL-4 on IL-1-induced IL-6 production in HGF and HPLF was very weak, but it was reliable. More studies may be required to determine this effect in HGF or HPLF.

In this study we found that HGF and HPLF produced IL-6 when treated with rhIL-1α, rhIL-1β or rhTNF-α, and the cytokine-induced IL-6 production was downregulated by endogenous PGE2. IL-6 induced by treatment with these cytokines enhanced its own production in the presence of IL-6sR. IFN-γ enhanced, but IL-4 slightly suppressed, rhIL-1β-induced IL-6 production. DEX markedly suppressed rhIL-1 (α and β) and rhTNF-α-induced IL-6 production. On the basis of this we suggest that in inflamed periodontal tissues, human gingival or periodontal ligament fibroblasts may be stimulated with pro-inflammatory cytokines to produce IL-6, which can be differentially modulated by endogenous PGE2, endogenous IL-6 in the presence of IL-6sR, T cell-derived cytokines such as IFN-γ or IL-4 and glucocorticoids.

Acknowledgement

This work was supported in part by grants-in-aid (04771534 and 06771745) from the Ministry of Science, Education and Culture of Japan.

References

Regulation of IL-6 production in fibroblasts


