Profiles of cytokine expression in radicular cyst-lining epithelium examined by RT-PCR

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Abstract: Levels of messenger RNA (mRNA) extracted from five samples of radicular cyst-lining epithelium were analyzed for cytokines, growth factors and epithelial cell growth-related receptors by RT-PCR. All five samples expressed IL-1α, -1β, IL-6, IL-8, IL-11, TGF-β1, PDGF-A and aFGF, and receptors for EGF (c-erbB), KGF, HGF (c-met) and IL-6. Some of the specimens expressed MIP-1α, RANTES, GM-CSF, M-CSF, TNF-α, PDGF-B and bFGF, but no expression of IL-2, IL-4, IFN-γ, IGF-I, EGF and KGF was detected. These results indicate that radicular cyst-lining epithelium, which is considered to be identical to the cell rests of Malassez, may play a role in periodontal pocket formation or apical cyst formation by interaction with surrounding connective tissue or hematopoietic cells through the expression of various cytokines. (J. Oral Sci. 42, 239-246, 2000)

Key words: radicular cyst-lining epithelium; cytokine; growth factor; growth factor receptor expression.

Introduction

Certain forms of stimulation of marginal or apical periodontal tissue may induce proliferation of the epithelial cell rests of Malassez, causing cell spreading in order to maintain epithelial continuity for covering the surface of connective tissue (1-10). Because tooth eruption breaks epithelial continuity, the tooth surface is the only part of the body that lacks an epithelial covering. If the marginal epithelial pegs migrate in an apical direction and extend between the periodontal ligament and the tooth, loss of connective tissue attachment, as seen in periodontitis, occurs (1,6-10). When the epithelium proliferates in the apical area, it forms radicular cyst-lining epithelium, as seen in apical cysts (5). Epithelial cells secrete matrix metalloproteinase (11,12), together with certain cytokines which accelerate collagenase secretion by surrounding fibroblasts (13-15). Therefore, clarification of the interaction between epithelium and the surrounding connective tissue or hematopoietic cells is very important for understanding the development and progression of oral inflammatory diseases such as periodontal disease and radicular cyst.

In an attempt to determine whether the epithelial cell rests forming a continuous network around the root (7-9) play a role in periodontal pocket or radicular cyst formation in combination with cytokines, we examined the mRNA expression of inflammatory cytokines, growth factors and epithelial cell growth-related receptors using reverse transcriptase-polymerase chain reaction (RT-PCR).

### Materials and Methods

**Cyst-lining epithelium biopsy**

Biopsy samples from five radicular cysts were obtained by surgical removal. All the patients gave their informed consent before providing the samples. A portion of each sample was fixed for routine histology, and the remainder was subjected to enzymatic digestion (4°C overnight) with 2,000 U/ml dispase (Godo Shusei) containing Dulbecco’s modified Eagle medium (DMEM, Iwaki) supplemented with 10% (v/v) fetal bovine serum (FBS, Iwaki) and antibiotics (50 U/ml penicillin, 50 μg/ml streptomycin, Sigma), in order to separate the epithelium from the granulation tissue and connective tissue of the cyst wall (15). Each detached sample of epithelium was further incubated in 5 ml of DMEM supplemented with 10% FBS and antibiotics at 37°C in 5% CO2/95% air for 6 h, after which RNA extraction was performed.

**Light microscopy**

Some of the detached epithelial strips were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at a thickness of 6 μm. The tissue was stained with hematoxylin and eosin using standard procedures.

**RT-PCR**

Total RNA was extracted from each sample of cyst epithelium using Trizol (Gibco, Life Technologies), according to the manufacturer’s protocol. The harvested RNA was then reverse-transcribed and amplified using a GeneAmp RNA PCR kit (Perkin Elmer). The cDNA was

### Table 1 Specific primers for the polymerase chain reaction

<table>
<thead>
<tr>
<th>gene</th>
<th>primer</th>
<th>product size (base pairs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>5’ CAAGGACACCAGATGTTAGTGAAGCAACAG</td>
<td>324</td>
</tr>
<tr>
<td>IL-1α</td>
<td>3’ TAGGCGTTCAGTGCAGG</td>
<td>307</td>
</tr>
<tr>
<td>IL-1β</td>
<td>5’ ATGCCGAGATCACCTAACAG</td>
<td>802</td>
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<tr>
<td>IL-1β</td>
<td>3’ ACACACTTGTGTAACGGTT</td>
<td>295</td>
</tr>
<tr>
<td>IL-2</td>
<td>5’ CAGCTGAGTCATCTGCTG</td>
<td>295</td>
</tr>
<tr>
<td>IL-2</td>
<td>3’ CAGCTGAGTCATCTGCTG</td>
<td>307</td>
</tr>
<tr>
<td>IL-4</td>
<td>5’ AGCGCTGTGAAATTTTCTCATT</td>
<td>307</td>
</tr>
<tr>
<td>IL-6</td>
<td>5’ AATTCGTCGATCTGCTGAG</td>
<td>307</td>
</tr>
<tr>
<td>IL-6</td>
<td>3’ CAGGACGTGAGTCAACGATT</td>
<td>307</td>
</tr>
<tr>
<td>IL-8</td>
<td>5’ CAGCTGAGTCATCTGCTG</td>
<td>307</td>
</tr>
<tr>
<td>IL-8</td>
<td>3’ TCACTCTGAGACAGATCGAGA</td>
<td>307</td>
</tr>
<tr>
<td>IL-11</td>
<td>5’ CTCTACAGCTCCAGGACTGTG</td>
<td>307</td>
</tr>
<tr>
<td>IL-11</td>
<td>3’ CAGTCAAGTCTAGTGAACGG</td>
<td>307</td>
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<tr>
<td>MEP-1α</td>
<td>5’ ACCCTGTCGTGGATCTGCTCA</td>
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<tr>
<td>MEP-1α</td>
<td>3’ TTTAAGAAGACCGTGCACTG</td>
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<tr>
<td>RANTES</td>
<td>5’ TCACTGAGTCATCTGCTG</td>
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<td>RANTES</td>
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<td>3’ AAGTACGGAGGATGCAAAGAAG</td>
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<td>M-CSF</td>
<td>5’ CAAGTCAGTGGAGAGATCGG</td>
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<tr>
<td>M-CSF</td>
<td>3’ TCTCTTCAGTACGGACACG</td>
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</tr>
<tr>
<td>TNF-α</td>
<td>5’ TCTGGGCTGAGTGAAGTAAAC</td>
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<tr>
<td>TNF-α</td>
<td>3’ TATCTCCTAAGCTCCACAGCA</td>
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</tr>
<tr>
<td>IFN-γ</td>
<td>5’ CAGGCTGAGTCATCTGCTG</td>
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</tr>
<tr>
<td>IFN-γ</td>
<td>3’ GCCTTCTGCAAGTCGACTCG</td>
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**TGF-β1** | 5’ GCCCTGGAACACACACTGTGCT | 161 |
**TGF-β1** | 3’ AGGCCTCAGACATGAGG | 161 |
**KIF-1** | 5’ ACATCTCCATCCTCCTGAGTTTCCTTTCG | 514 |
**KIF-1** | 3’ CCCTACTTCTGTCTTCAAAATGACTTAC | 514 |
**PDGF-A** | 5’ AGAAATCTCGGAAATGTTGAGGAGACAT | 304 |
**PDGF-A** | 3’ CTCTACAGCTCCAGGACTGTGACTCAGCA | 497 |
**PDGF-B** | 3’ CTTCCTGAGGACTGAAATGACCTGACATTGTGAC | 497 |
**PDGF-B** | 3’ GCCCTGGAACACACACTGTGCT | 489 |
**afGIF** | 5’ GCTGGCACTGAGGCAAA | 489 |
**afGIF** | 3’ ACATCTTCTTAAACTCAAAAGGAATG | 489 |
**NGF** | 5’ GAGATGCTGCTGAGTGAAGTAAAC | 243 |
**NGF** | 3’ TCACTGAGTCATCTGCTG | 243 |
**EGF** | 5’ CTCTACAGCTCCAGGACTGTG | 240 |
**EGF** | 3’ GCCCTGGAACACACACTGTGCT | 240 |
**KGF** | 5’ TCTCTGCTGAGTGAAGTAAAC | 266 |
**KGF** | 3’ TCTCTTCTGTCTCCATACGACG | 266 |
**c-erbB** | 5’ GCTGGGAACACACTGTGCT | 259 |
**c-erbB** | 3’ CTCTTCTGAGTGAAGTAAAC | 259 |
**KGF-R** | 5’ CTCTTCTGAGTGAAGTAAAC | 175 |
**KGF-R** | 3’ TCTCTTCTGAGTGAAGTAAAC | 175 |
**c-met** | 5’ GCTGGCACTGAGGCAAA | 342 |
**c-met** | 3’ CTCTTCTGTCTCCATACGACG | 342 |
**IL-6R** | 5’ TCTGGGCTGAGTGAAGTAAAC | 394 |
**IL-6R** | 3’ TCTGGGCTGAGTGAAGTAAAC | 394 |
**β-actin** | 5’ AGGGCCCGACCTGCGTACTC | 621 |
**β-actin** | 3’ CACTCTGCCATCCACAGAG | 621 |
amplified using specific primers for various cytokines and receptors, as shown in Table 1, in a reaction mixture containing Taq polymerase. PCR was performed in a DNA thermal cycler (Perkin Elmer) for 35 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C. The amplified samples were visualized on 2% agarose gels stained with ethidium bromide and photographed under UV light. The RNA from a well characterized gingival epithelial cell line (Ca9-22) (16-18) was used as a control because this is the only established epithelial cell line derived from periodontium.

Results

Histology
Histologic observation revealed that the separated cyst epithelia were composed of stratified squamous epithelium (Fig. 1).

Cytokine expression
The expression of mRNA for several cytokines from the cyst-lining epithelium is shown in Fig. 2. All the epithelial samples expressed IL-1α, IL-1β (except case 3), IL-6 and IL-8 strongly, and expressed IL-11 weakly. Similar results, except for strong IL-11 and weak IL-8 expression, were obtained for a control cell line (Ca9-22).

One to four of the epithelial samples expressed MIP-1α, RANTES, GM-CSF, M-CSF and TNF-α to various degrees, whereas Ca9-22 cells did not express MIP-1α, RANTES or TNF-α.

However, expression of IL-2, IL-4 or IFN-γ was not observed in any of the samples, or in Ca9-22 cells (data not shown).

Growth factor expression
The expression of growth factor mRNA is shown in Fig. 3. All the samples of cyst-lining epithelium, and the Ca9-22 cells, expressed TGF-β1 strongly, and expressed PDGF-A and αFGF moderately.

One of the five epithelial samples and the Ca9-22 cells expressed PDGF-B; three of the samples expressed bFGF, but Ca9-22 cells did not.

No expression of IGF-1, EGF or KGF was detected in
any of the samples or in the control (data not shown).

Expression of epithelial cell growth-related receptor

The mRNA expression of some growth factor receptors is shown in Fig. 4. As major growth factor receptors for epithelial cells, EGFR (EGF receptor, c-erbB), KGFR, HGFR (c-met) and IL-6R were expressed in all the samples of cyst epithelium examined, and also in Ca9-22 cells.

Discussion

Cytokine expression of epidermal keratinocytes has been extensively investigated during the past decade, and there is increasing evidence that these cytokines are physiologically involved in homeostasis of the skin (19). Furthermore, cytokines produced by epidermal keratinocytes are regarded as important regulators/mediators of the inflammatory process, immune responses and wound healing (20-22). Dysfunction at the cytokine level contributes to several diseases of the skin (23,24). In contrast to the epidermis, only a few studies have investigated cytokine expression profiles in the oral epithelium under normal and pathological conditions (25).

Once the epithelium has been injured, epithelial cells become activated, and migrate towards the wound to cover the surface defect (10,19-22). If this normal defense system were to act around the tooth, it would induce loss of the connective tissue attachment between the tooth and alveolar bone, as observed in periodontitis or periapical cyst formation (1,5-10). Therefore, interaction between epithelium and connective tissue or hematopoietic cells is of special importance for understanding the development of periodontal pockets or radicular cysts in this unique tissue.

The present study revealed that samples of cyst-lining epithelium, and a control cell line, strongly expressed both IL-1α and IL-1β mRNA. Our previous study demonstrated that gingival epithelial cells secreted an IL-1α-like factor which stimulated collagenase production by periodontal ligament cells and gingival fibroblasts (14). Furthermore, we found that the cyst-lining epithelium secreted a substance which accelerated the production of collagenase by periodontal ligament fibroblasts (15). This would be one of the reasons why epithelial cells show apical migration through epithelial-stromal interaction. Other than inducing fibroblast collagenase production, IL-1 has multiple effects on immunocompetent cells as a pro-inflammatory cytokine (26). Furthermore, IL-1 is a potent inducer of bone resorption in jaw cysts (27), and histological studies have revealed that the cyst-lining epithelium expresses IL-1 extensively (28-30). These observations suggest that expression of IL-1 mRNA is intrinsically involved in the formation of periodontal or apical lesions.

Other cytokines involved in bone resorption, IL-6 and IL-11, were also expressed in the cyst-lining epithelium. It has been reported that IL-6 is expressed in cyst-lining epithelium (30) and peritonsillar mucosa (31). Expression of IL-11 in the control cell line was stronger than that in the samples of cyst-lining epithelium. Although IL-11 expression by alveolar A549 cells and airway 9HTE cells has already been reported by Elias et al. (32), the present study appears to be the first in which expression of IL-11 has been identified in oral epithelial cells. Together with
IL-1, these cytokines would facilitate bone resorption or B-cell activation (33). IL-8 is known to act as a chemoattractant for PMN (34). Li et al. (35) reported that oral keratinocytes secrete IL-8 and RANTES when stimulated with TNF-α and/or IFN-γ. Chemokine expression in oral mucosa and keratinized mucosa has also been reported by Zehnder et al. (36) and Bickel et al. (37), respectively. These cytokines would act as major chemoattractants for PMN in cyst-lining epithelium, similarly to oral keratinocytes. In fact, PMN has been detected in gingival crevicular fluid (38) and radicular cyst fluid (39).

Other cytokines expressed in some cases, such as MIP-1α (40,41), RANTES (35), GM-CSF and M-CSF (20), would act as chemoattractants for immunocompetent cells. It has been reported that oral keratinocytes are induced to produce RANTES by TNF-α and IFN-γ synergistically, whereas they produce IL-8 when exposed to TNF-α alone (35). This difference is one of the reasons why IL-8 expression was observed in all of the present specimens, whereas RANTES expression was observed in only two. TNF-α (30) can act as a pro-inflammatory cytokine, and stimulate bone resorption (42) like IL-1, IL-6 and IL-11. These cytokines might be inducible rather than constitutive when epithelial cells receive various kinds of stimulation. Similarly, some of the cytokines whose expression was not observed in the control cell line, such as MIP-1α, RANTES, TNF-α and bFGF, would be inducible.

Honma et al. (30) reported that expression of mRNA for IL-1α, IL-1β, IL-6, IL-8 and TNF-α was detectable by in situ hybridization in radicular cyst epithelium. Our present study using RT-PCR confirmed their results, although the findings for TNF-α were slightly different.

Growth factors such as TGF-β1, PDGF-A and aFGF were also expressed in cyst-lining epithelium, as reported previously for oral keratinocytes (25). These factors are known to accelerate wound healing by stimulating fibroblasts or other cells (43-45). Expression of TGF-β1 in the junctional and oral gingival epithelium has also been reported by Lu et al. (40). Expression of these factors would provide feedback regulation of inflammatory cytokines. In fact, Steinsvoll et al. (41) reported marked expression of TGF-β1 in pocket epithelium in lesions with extensive inflammation, and suggested that upregulation of TGF-β1 in the inflamed gingiva may counterbalance the destructive gingival inflammatory responses that occur simultaneously in patients with chronic marginal periodontitis.

Other cytokines expressed in some of our specimens, such as PDGF-B (44) and bFGF (45), would regulate fibroblast metabolism on certain occasions when exposed to some forms of stimulation.

No expression of IL-2, IL-4 or IFN-γ was observed, as reported elsewhere (25,31), and this suggested that our samples were T-cell-free. Furthermore, KGF expression was not detected either, indicating that fibroblasts may have been eliminated from the samples.

This study also demonstrated expression of receptors for EGF, KGF, HGF and IL-6. Previous studies have revealed that the epithelial cell rests of Malassez express EGF receptors and their role in the genesis of dental cysts has been suggested (46,47). IL-6 is also known to act as an autocrine growth factor for epithelial cells (48).

Recent investigations have revealed that KGF is a potent mitogen for epithelial cells produced by fibroblasts (49,50), and Gao et al. (51) found KGF-expressing fibroblasts just beneath the proliferating epithelium in apical lesions. HGF receptor (c-Met protein) and its ligand, HGF, has received attention because of its multifunctional characteristics for epithelial cells including matrix invasion and cancer metastasis through its activity as a scatter factor (52). We hypothesized that this factor might induce apical migration of the epithelium around the root because fibroblast-conditioned medium treated with anti-HGF neutralizing antibody lost a significant amount of its chemotactic activity for the gingival epithelial cell line, Ca9-22 (53). For these reasons, the expression of KGFR and HGFR (c-met) not only in the control cell line but also in the cyst-lining epithelium in the present study is of considerable interest.

Since it is reported that IL-1 stimulates fibroblast KGF or HGF production (54), secretion of IL-1 by the cyst-lining epithelium would stimulate the underlying fibroblasts to secrete KGF and HGF, thus inducing apical migration and proliferation of epithelial cells to maintain epithelial continuity.

Further study is required to clarify the relationship between the epithelial cell rests of Malassez and periodontal pocket or periapical cyst formation on the basis of the cytokine expression profiles revealed in this investigation.

Acknowledgments

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