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Gingival fibroblasts from periodontitis patients exhibit inflammatory characteristics *in vitro*

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ABSTRACT

Objective: Gingival fibroblasts (GFs) are an important regulatory cell type in the progression of periodontitis. This study aimed to compare the expression levels of genes associated with inflammation, extracellular matrix degradation and bone destruction in GFs isolated from healthy and periodontitis subjects in the absence and presence of *Porphyromonas gingivalis*.

Designs: Primary GFs from healthy ($n = 10$) and periodontitis subjects ($n = 10$) were stimulated *in vitro* with viable *P. gingivalis* ATCC 49417 and 3 clinical isolates of *P. gingivalis* with type II fimbriae from one healthy subject (KUMC-H1) and two periodontitis patients (KUMC-P1, -P2). The mRNA expression of proinflammatory cytokines (interleukin (IL)-6, IL-8, IL-1B), anti-inflammatory cytokines (IL-4, IL-10), matrix metalloproteinase (MMP)-1 and 2, tissue inhibitor matrix metalloproteinase (TIMP)-3 and osteoprotegerin (OPG) were assessed using real-time PCR. The levels of IL-6, IL-1β and TIMP-3 protein were measured by an enzyme-linked immunosorbent assay.

Results: The mRNA expression of IL-6, IL-1B and TIMP-3 was higher in the periodontitis group compared with the healthy group, whereas IL-4 expression was higher in the healthy group both in the absence and presence of the *P. gingivalis* strains. The expression levels of IL-6, IL-1β and TIMP-3 protein were also higher in the periodontitis group in the absence and/or presence of the *P. gingivalis* strains. There was inter-strain variability among *P. gingivalis* strains in the ability to induce expression of the proinflammatory cytokines, MMPs and OPG and in the ability to degrade IL-6 protein.

Conclusion: High expression of proinflammatory cytokines and TIMP-3 and low expression of IL-4 can be a signature of GFs associated with periodontitis.

1. Introduction

Inflammation, extracellular matrix degradation and alveolar bone destruction are crucial events in the progression of periodontitis. Although a number of gram-negative anaerobic bacteria are required in the initiation of periodontitis, inflammation and tissue destruction are believed to be the result of the host response to the bacteria and their products.[1]

Inflammation and tissue destruction is aggravated by several destructive factors, but it can be attenuated by counteraction with protective factors. Host proinflammatory cytokines are known to be associated with tissue destruction, whereas anti-inflammatory cytokines possess suppressive and anti-inflammatory properties.[2] The intensity of interleukin

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(IL)-6 and IL-1β expression is positively correlated with attachment loss,[3,4] and these cytokines are associated with continuous tissue destruction in periodontitis.[5] As opposed to the destructive pathway triggered by proinflammatory cytokines, the anti-inflammatory cytokines IL-4 and IL-10 have marked suppressive and anti-inflammatory properties, which are mediated by their capacity to inhibit the transcription of proinflammatory cytokines.[2] Decreasing levels of IL-4 are correlated with loss of collagen and with increasing clinical severity of periodontitis,[6] and the concentration of IL-4 in gingival crevicular fluid was decreased from periodontal health to disease.[7] The connective tissue destruction in periodontitis is caused by matrix metalloproteinase (MMP) expression induced on host cells that are stimulated by periodontopathogens.[8] MMPs are believed to be the most important factors in gingival connective tissue destruction.[8] Tissue inhibitors of matrix metalloproteinases (TIMPs) inhibit the effect of MMPs on tissue destruction, and the extracellular matrix destruction in periodontitis is caused by the breakdown of the balance between MMPs and TIMPs.[8] Bone resorption in periodontitis occurs *via* the concerted action of osteoclast-stimulating or osteoclast-inhibiting mediators. Receptor activator of nuclear factor-kappa B ligand (RANKL) stimulates bone resorption, whereas osteoprotegerin (OPG) blocks its action.[9] Interestingly, the destruction of the extracellular matrix and the alveolar bone by periodontitis is accelerated by proinflammatory cytokines, such as IL-6 and IL-1β, but inhibited by anti-inflammatory cytokines, such as IL-4 and IL-10, through modulation of both the MMPs/TIMPs and RANKL/OPG systems.[2] Collectively inflammation, extracellular matrix and bone destruction are accelerated by proinflammatory cytokines, MMPs and RANKL but attenuated by anti-inflammatory cytokines, TIMPs and OPG.

Gingival fibroblasts (GFs) are the predominant cell type in periodontal connective tissue. GFs produce components of the extracellular matrix and participate in the regulation of turnover of the extracellular matrix through the production of MMPs and TIMPs.[10] Moreover, the ability of GFs to induce the expression of RANKL and OPG shows that GFs can participate in the formation of osteoclasts.[11] Recently, it has been demonstrated that GFs can also participate in the immune response against bacteria. GFs constitutively expressed several pattern recognition receptors, such as toll-like receptors (TLRs) 1–9 and nucleotide-binding oligomerization domain proteins, which are functional receptors involved in the inflammatory reaction.[12] Taken together, these findings indicate that GFs may be an important regulatory cell type in the progression of periodontitis by regulating inflammation, matrix degradation and bone destruction.

Porphyromonas gingivalis is a major periodontopathic bacterium.[13] It can penetrate the epithelial barrier, enter the connective tissue and stimulate several cells in the connective tissue to induce an inflammatory response.[14,15] *P. gingivalis* has been suggested to possess clonal heterogeneity because it can be present in both periodontal pockets undergoing destruction and the healthy gingival sulcus.[16,17] Genotyping studies have supported genetic variability among *P. gingivalis* strains.[18–20] It is generally accepted that laboratory strains are not necessarily comparable to clinical strains, either in virulence or in metabolic function; thus the analysis of *P. gingivalis* laboratory strains alone is not likely to be sufficient to study the interaction between the bacteria and the host cell.[21] Although there have been many studies that examined the effect of *P. gingivalis* on the expression of genes associated with the progression of periodontitis by GFs *in vitro*, the effect of both the lab strain and clinical isolates remains unclear.[10,11,22]

Recent studies have shown that GFs from different individuals have considerable heterogeneity. GFs from different individuals showed different responses to *P. gingivalis* in their collagen-degrading ability and the expression of cytokines and chemokines.[10,22] We hypothesized that the GFs from healthy and periodontitis subjects would show a distinct phenotypic response due to underlying genotypic differences: GFs from periodontitis patients would show higher expression of destructive factors associated with the progression of periodontitis compared with GFs from healthy subjects. To verify this hypothesis, this study compared the expression levels of destructive and protective genes associated with inflammation, extracellular matrix degradation and bone destruction by GFs obtained from healthy and periodontitis subjects in the absence and presence of viable *P. gingivalis* strains. This study utilized not only the lab strain but also clinically isolated *P. gingivalis* strains to determine whether there is inter-strain variability among the *P. gingivalis* strains in the ability to induce genes from GFs.

2. Materials and methods

2.1. Human subjects and clinical assessments

A total of 20 adult subjects, who sought dental treatment at the Anam Hospital of Korea University, were recruited for the study. The study protocol was approved by the Institutional Review Board for Human Subjects of the Korea University Anam Hospital (IRB No. AN10053-001). Written informed consent was obtained from all individuals. Exclusion criteria included smoking, pregnancy, diabetes, and other systemic conditions that could affect the periodontal status. Probing depth (PD) was performed at 6 sites/teeth. The amount of marginal alveolar bone loss was estimated to be the percentage of the length from the cement–enamel junction to the alveolar crest relative to the length from the cement–enamel junction to root apex on panoramic view. The periodontal healthy group consisted of subjects exhibiting no sites with PD > 4 mm and no marginal bone loss $> 20\%$; the chronic periodontitis group consisted of subjects exhibiting at least two or more sites with PD ≥ 5 mm on a quarter jaw and three or more teeth with marginal bone loss $> 20\%$ on one jaw.

2.2. Gingival fibroblasts

Primary GFs were obtained from 10 healthy subjects and 10 periodontitis patients. The GFs from healthy controls were cultured from explants of gingiva from patients undergoing third molar extraction or a crown-lengthening procedure. The periodontitis patients preferentially received scaling and root debridement to remove gingival inflammation, and the GFs from periodontitis donors were obtained from explants of gingiva from patients undergoing a modified Widman flap surgery. The GFs were obtained from explants of gingiva