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Review Article

The human periodontal ligament cell: a fibroblast-like cell acting as an immune cell

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Background: Periodontal ligament cells are fibroblast-like cells characterized by collagen production but also possessing some osteoblastic features. In the light of numerous studies presented during recent times, which show that human periodontal ligament cells also produce cytokines and chemokines in response to inflammation promoters, it is reasonable to suggest that periodontal ligament cells play a role as promoters of periodontal inflammation through these mechanisms.

Material and Methods: The periodontal ligament, which harbours the periodontal ligament cells, is a part of the attachment apparatus comprised of periodontal ligament cells, extracellular matrix and fibres, attaching the root cement to the alveolar bone. Periodontal ligament cells are in close proximity to bacteria within the plaque and the pocket, and thus these cells are readily accessible to bacterial endotoxins and other promoters of inflammation.

Results: Cytokines and chemokines, released by periodontal ligament cells upon stimulation with inflammation promoters, reach the blood vessels easily thanks to rich vascularization of the periodontium stimulating recruitment of white blood cells to the site of inflammation. In addition to classical inflammatory cells, such as leucocytes, macrophages and mast cells, the periodontal ligament cells also contribute to periodontal inflammation via their production and release of cytokines and chemokines.

Conclusion: Therefore, pharmacological treatment of periodontitis should aim to reduce the release of proinflammatory agents not only from classical inflammatory cells but also from periodontal ligament cells.

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The process of generating human periodontal ligament cells

The human periodontal ligament cells used in experimental studies are obtained by allowing cells to grow out of periodontal ligament tissue explants kept under growth-stimulating conditions (cell culture medium containing 10% foetal calf serum as growth promoter). This protocol was established by Somerman *et al.* (1,2), and it has been used in numerous studies. The explants are scraped off the middle third of the root surface to avoid contamination with marginal or apical tissues. The teeth used for collecting explants are normally extracted for orthodontic reasons from boys and girls between 12 and 18 years of age. The periodontal ligament cells start to grow out from the explants within about 10 d. The viability of the cells is high, probably due to the fact that the cells originate from young and healthy individuals. After passage (confluent cells trypsinized in 0.25% trypsin), the cells are re-seeded and then used for experiments in passages two to eight. In passages three to

five, the cells respond in a similar manner to stimulation with hormones and growth promoters (3), suggesting that their phenotype is maintained, although some osteogenic markers, e.g. alkaline phosphatase activity, have been shown to be decreased at later (passage 6) compared with earlier passages (4). The periodontal ligament cells in passages three to five show a fibroblast-like morphology, with a spindle-shaped cell form (5). Cell viability decreases at later passages (beyond passage 7), suggesting that the periodontal ligament cells then go into senescence (2).

Periodontal ligament cells produce collagen and show some osteoblast-like features

The periodontal ligament cells produce and secrete extracellular matrix components, such as collagen, building up the periodontal ligament and its fibres, to secure attachment of root cement to alveolar bone and to allow regeneration of the periodontal ligament to occur upon injury. Indeed, periodontal ligament cells have a high production of collagen (1,6). Besides producing collagen, periodontal ligament cells may also produce mineralized tissue. Human periodontal ligament cells have been shown to express high levels of alkaline phosphatase activity and bone-associated proteins, such as osteonectin, and to form mineralized nodules, suggesting that periodontal ligament cells are osteogenic cells (2,7). The periodontal ligament cells do not, however, respond to calcitonin or parathyroid hormone and they do not express other bone marker proteins, such as bone sialoprotein 1, in contrast to osteoblasts (2). Piche *et al.* (8) showed that periodontal ligament cell clones derived from periodontal ligament explants obtained from two different individuals exhibited high and low basal alkaline phosphatase activity, respectively, suggesting a functional heterogeneity between periodontal ligament cell clones originating from different individuals. Taken together, these data show that the periodontal ligament cells do not fully behave as classical osteoblasts. Human periodontal ligament cells and gingival fibroblasts show many functional similarities, but they also possess different functional characteristics, e.g. high alkaline phosphatase activity is detected in periodontal ligament cells but not in gingival fibroblasts (9).

Heterogeneity of periodontal ligament cells in culture

Periodontal ligament cells in culture possess many diverse cell-phenotype characteristics, e.g. those typical for fibroblasts, such as fibroblast morphology and collagen production (1,3,6), and those typical for osteocytes and osteoblasts, such as alkaline phosphatase activity and expression of bone-associated proteins (2,7). It is possible that a subpopulation of periodontal ligament cells showing osteogenic properties is derived from bone cells dislodged into the periodontal ligament during extraction. Although cultured periodontal ligament cells respond in a similar manner to hormones and growth factors in passages three to five (3), suggesting that they represent a rather homogeneous and stable population of cells, we cannot rule out the possibility that periodontal ligament cells in culture represent multiple cell types. The stimulation of periodontal ligament cell production of cytokines and chemokines by inflammation promoters described below is a consistent and highly reproducible periodontal ligament cell response to this type of stimulation, probably representing a global property of periodontal ligament cells in culture, although we cannot completely rule out the possibility that this response is associated only with a subpopulation of cultured periodontal ligament cells.

Inflammation promoters stimulate periodontal ligament cell cytokine and chemokine mRNA and protein production

Recent studies suggest that human periodontal ligament cells, in addition to their fibroblastic and osteoblastic properties, also possess functional characteristics similar to those of leucocytes and leucocyte-derived cells (e.g. macrophages) involved in classical innate immunity. The periodontal ligament cells have been shown to express and produce cytokines and chemokines in response to inflammation promoter stimulation, as shown both at the mRNA and at the protein level (see Table 1 for references). In unstimulated human periodontal ligament cells, cytokine and chemokine transcript and protein levels are low or below the limit of detection, but they increase several-fold upon stimulation with inflammation promoters, such as bacterial lipopolysaccharide (LPS). Lipopolysaccharide also induces cytokine production in human gingival fibroblasts (10,11), suggesting that periodontal ligament cells and gingival fibroblasts act together to promote proinflammatory actions. Cytokine and chemokine production by periodontal ligament cells is observed in response to stimulation with low, intermediate and high concentrations (1 ng/mL to 10 μg/mL) of LPS (12,13). Lipopolysaccharide-induced periodontal ligament cell cytokine/chemokine expression is observed within hours but also after several days (3–21 d) of treatment, showing that both acute and long-term stimulation with inflammation promoters activate cell-signalling pathways leading to cytokine/chemokine production (12).

Inflammation promoter-induced cytokine and chemokine production is observed in experiments performed in periodontal ligament cells in passages three to five, i.e. in cells which have been trypsinized several times and cultured for many weeks from the time point at which the cells start to grow out of the periodontal ligament tissue explants (about 10 d after tooth extraction and seeding of explants). Lipopolysaccharide-induced cytokine and chemokine production in primary periodontal ligament tissue explants and in cells derived directly from the explants (i.e. at short time points) may be associated with ordinary inflammatory cells that happen to be scraped off the tooth, but the stimulation of cytokine/chemokine production by LPS observed in cells at passages three to five is most probably due to stimulation of long-lived and persistent cells, such as classical periodontal ligament fibroblasts. It is also important to conclude that both acute

Table 1. A review of papers presenting data on proinflammatory stimulus-induced cytokine/chemokine expression in human periodontal ligament cells.

Reference	Proinflammatory stimulus	Cytokine/chemokine	Transcript/protein level
Jönsson *et al*. (12)	*Escherichia coli* LPS	IL-6, MCP-1	Protein
Jönsson *et al*. (13)	*E. coli* LPS	GROα	mRNA and protein
Okada *et al*. (19)	TNF-α	IL-6	Protein
Yamamoto *et al*. (29)	*Porphyromonas gingivalis, P. intermedia*	IL-1β, IL-6, IL-8	mRNA
Ogura *et al*. (30)	*P. endodontalis* LPS	IL-6	mRNA and protein
Agarwal *et al*. (31)	*Actinobacillus actionomycetem-comitans* LPS, *E. coli* LPS	IL-1β, IL-6, IL-8	mRNA and protein
Shu *et al*. (32)	*E. coli* LPS	TNFα, IL-1β, IL-6, RANKL	mRNA and protein
Yamaji *et al*. (33)	*P. gingivalis* LPS, *E. coli* LPS	IL-6, IL-8	mRNA and protein
Engels-Deutsch *et al*. (34)	*Streptococcus mutans*	IL-6, IL-8	Protein
Ozaki *et al*. (35)	TNFα, IL-1β	MCP-1	mRNA and protein
Lee *et al*. (36)	Reactive oxygen species (H_2O_2)	IL-8	mRNA and protein
Morandini *et al*. (37)	*P. gingivalis* LPS	IL-6	Protein
Wada *et al*. (38)	*E. coli* LPS	IL-1β, TNFα	mRNA

(24 h) and chronic (3–21 d) LPS stimulation of periodontal ligament cells seeded at passages three to five enhances cytokine and chemokine production several-fold (12).

Mechanisms behind inflammation-promoter-induced periodontal ligament cell cytokine and chemokine expression

Lipopolysaccharide binds to its receptor, the Toll-like receptor 4 (TLR4), and this complex regulates gene transcription of cytokine and chemokine genes via different adaptor proteins and transcription factors (14,15). Toll-like receptor 4 signalling involves a MyD88-dependent as well as a MyD88-independent pathway causing activation of nuclear factor-κB (NF-κB). The MyD88-independent signalling involves activation of the adaptor molecule TRAM, which forms a complex with another adaptor molecule named TRIF. This complex then binds the adaptor protein tumor necrosis factor receptor associated factor 6 (TRAF6), leading to activation of NF-κB. Both the MyD88-dependent and the MyD88-independent activation of NF-κB induce expression of inflammatory genes. Thus, NF-κB is a possible drug target for anti-inflammatory treatment. In a murine periodontal ligament cell line, Patil *et al*. (16) have shown that *Actinobacillus actinomycetemcomitans* and *Escherichia coli* LPS-induced IL-6 expression is dependent on multiple MAPK pathways, including ERK and c-jun N-terminal kinase (JNK), demonstrating that many intracellular signalling pathways regulate periodontal ligament cell cytokine gene activity.

Glucocorticoids and human periodontal ligament cells

The glucocorticoids bind to the nuclear glucocorticoid receptor to form a ligand–receptor complex. This complex is supposed to attenuate inflammation by inhibition of NF-κB-dependent transcription of proinflammatory genes (17). Dexamethasone is the most widely used glucocorticoid in biological experiments. Human periodontal ligament cells accumulate radioactive ([^{3}H]-labelled) dexamethasone, suggesting that periodontal ligament cells express the glucocorticoid receptor (18). Treatment with dexamethasone has been shown to reduce tumour necrosis factor-α-induced interleukin-6 and interleukin-8 production (19,20) and to reduce LPS-induced chemokine ligand 1 (GROα) chemokine expression (13) in human periodontal ligament cells. Besides suppressing cytokine/chemokine production via inhibition of NF-κB, dexamethasone drives the periodontal ligament cells towards an osteoblastic phenotype (21). However, a selective response to dexamethasone, promoting the osteoblastic features of a subpopulation of osteoblast-like periodontal ligament cells, may represent an alternative mechanism by which dexamethasone affects periodontal ligament cells in culture. Although dexamethasone is supposed to reduce inflammation via a direct mechanism involving inhibition of NF-κB, the transition of periodontal ligament cells from a fibroblast-like to an osteoblast-like cell phenotype and/or the stimulation of a subpopulation of osteoblast-like cells by dexamethasone may indirectly reduce the periodontal ligament cell production of proinflammatory factors. It is reasonable to suggest that glucocorticoids may be used to block the unwanted production of periodontal ligament cell cytokines and chemokines, but the indirect effects of glucocorticoid treatment are many. Therefore, other more specific pharmacological approaches are probably necessary, e.g. through interactions with periodontal ligament cell MyD88 and/or MAPK signalling, to achieve inhibition of periodontal ligament cell cytokine/chemokine expression.

Proinflammatory activity of fibroblast-like cells other than periodontal ligament cells

The periodontal ligament cell is not the only fibroblast-like cell type that produces cytokines/chemokines upon

stimulation with inflammation promoters. Bronchial and nasal fibroblasts and airway smooth muscle cells respond to interleukin-1β, tumour necrosis factor-α, viral elements and mechanical strain by increased production of both cytokines and chemokines, a mechanism believed to be involved in the pathophysiology of allergic inflammation, airway inflammation and pulmonary fibrosis (22–25). Fibroblast-like cells within synovial membranes, such as synovial fibroblasts, produce collagen and other connective tissue molecules to form the joint capsule, but in rheumatoid arthritis the synovial fibroblasts are transformed into an invasive cell type that produces a wide range of cytokines and chemokines (26,27). Renal fibroblasts produce cytokines/chemokines in response to proinflammatory stimuli, such as LPS and tumour necrosis factor-α, a mechanism contributing to the process of renal fibrosis (28). In conclusion, proinflammatory stimuli induce cytokine and chemokine production in different populations of fibroblasts within many tissues and organs, representing a mechanism involved in the pathophysiology of inflammatory diseases.

Concluding remarks

The human periodontal ligament cell produces not only periodontal ligament extracellular matrix but also proinflammatory cytokines and chemokines upon stimulation with inflammation promoters. Therefore, the periodontal ligament cell most probably plays a significant role to initiate recruitment of leucocytes in periodontal inflammation. Thus, pharmacological anti-inflammatory treatment of periodontal disease should aim at reducing the periodontal ligament cell production of cytokines and chemokines. It is an interesting and thrilling proposal that periodontal ligament cells themselves are responsible for initiating periodontal inflammation via these mechanisms.

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