Cytokine 55 (2011) 98–103

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/issn/10434666

The RANKL–OPG system is differentially regulated by supragingival and subgingival biofilm supernatants

Georgios N. Belibasakis [a,*], Andre Meier [a], Bernhard Guggenheim [a], Nagihan Bostanci [b]

[a] Oral Microbiology and Immunology, Institute of Oral Biology, Center of Dental Medicine, University of Zürich, Plattenstrasse 11, 8032 Zürich, Switzerland
[b] Oral Translational Research, Institute of Oral Biology, Center of Dental Medicine, University of Zürich, Plattenstrasse 11, 8032 Zürich, Switzerland

ARTICLE INFO

Article history:
Received 8 November 2010
Received in revised form 4 February 2011
Accepted 15 March 2011
Available online 6 April 2011

Keywords:
Oral biofilms
Gingival fibroblasts
Receptor activator of NF-kβ ligand
Osteoprotegerin
Periodontitis

ABSTRACT

Periodontitis is an inflammatory condition that destroys the tooth supporting tissues, including the alveolar bone. It is triggered by polymicrobial biofilms attaching on tooth surfaces, which can be supragingival or subgingival. Bone resorption is triggered by receptor activator of NF-κB ligand (RANKL) and blocked by its soluble decoy receptor osteoprotegerin (OPG), which are cytokines of the tumor necrosis factor ligand and receptor families, respectively. The present study aimed to comparatively investigate the effects of the Zürich *in vitro* supragingival and subgingival biofilm models, on RANKL and OPG gene expression in primary human gingival fibroblasts (GF) cultures. The cells were challenged with biofilm culture supernatants for up-to 24 h. RANKL and OPG gene expression in the cells was analyzed by quantitative real-time polymerase chain reaction (qPCR) and their relative RANKL/OPG ratio was calculated. Both biofilm supernatants induced RANKL expression, but the subgingival caused a more pronounced up-regulation compared to the supragingival (10-fold at 6 h and 100-fold at 24 h). Changes in OPG expression in response to either biofilm were more limited. Accordingly, the subgingival biofilm caused a greater enhancement of the relative RANKL/OPG ratio (4-fold at 6 h and 110-fold 24 h). In conclusion, subgingival biofilms exhibit a stronger potency for inducing molecular mechanisms of bone resorption than supragingival biofilms, in line with their higher virulence nature for the development of periodontitis.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Periodontal diseases are perhaps the most common chronic inflammatory diseases in man. Their main trait is the inflammatory destruction of the tooth-supporting (periodontal) tissues, as a result of oral bacteria colonizing the tooth surfaces in the form of polymicrobial biofilm communities [1]. Depending on the localization of the biofilm in relation to the gingival margin, this can be either "supragingival" (above) or "subgingival" (below) [2]. Supragingival biofilms are typically constellated by Gram positive, facultative anaerobic and non-motile species, whereas subgingival biofilms are characterized by the dominance of Gram negative, anaerobic and motile species. Bacterial products released by the biofilms can cause an inflammatory response by the periodontal tissues, aiming to eliminate the bacterial challenge [3]. However, rather than being protective, an excessive inflammatory response induces periodontal tissue damage [4]. Gingivitis is a clinical condition in which the host-inflammatory response to the biofilm is restricted to the superficial gingival tissue and is typically associated with the presence of a supragingival biofilm. However, if the inflammation progresses into the deeper periodontal tissues, it is accompanied by a concomitant deepening of the gingival epithelium and the creation of a pathological pouch known as periodontal pocket. The niche of the periodontal pocket is an optimal environment for the colonization and growth of species that preferentially form subgingival biofilm communities [5]. The presence of subgingival biofilms is detrimental for the development of periodontitis, as the associated species are considered more pathogenic for this disease [1,2,6,7]. Periodontitis represents a progressive inflammatory switch from gingivitis that involves further the destruction of the alveolar bone, eventually leading to tooth loss.

Bone resorption in physiological and pathological conditions, such as periodontitis, is regulated by the interplay of a system of two cytokines belonging to the tumor necrosis factor ligand and receptor superfamilies. These are respectively receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG). RANKL is expressed by osteobasts, synovial or gingival fibroblasts (GF) and activated T- and B-cells. By activating its cognate RANK receptor on osteoclast precursors (cells of the monocyte/macrophage lineage), it triggers their fusion and differentiation into multinucleated osteoclasts, which are the bone-resorbing cells [8]. The soluble decoy receptor OPG can bind to RANKL, thus inhibiting

Abbreviations: RANKL, receptor activator of NF-κB ligand; OPG, osteoprotegerin; GF, gingival fibroblasts; LDH, lactate dehydrogenase; qPCR, quantitative real-time polymerase chain reaction.

* Corresponding author. Tel.: +41 44 6343396.
E-mail address: george.belibasakis@zzm.uzh.ch (G.N. Belibasakis).

1043-4666/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cyto.2011.03.009

the RANK–RANKL interaction and the downstream events that lead to bone resorption [9]. Changes in the relative RANKL/OPG ratio are indicative of the capacity of a cell or tissue to regulate bone resorption. An increased ratio is indicative of enhanced bone resorption in pathological inflammatory conditions, such as periodontitis [10,11].

The cells of the gingival connective tissue have an important role in the protection and homeostasis of the periodontium. GF constitute the main cell population of this tissue producing collagenous matrix, but also responding to bacterial challenge by producing mediators of inflammation [12]. They constantly express OPG but do not regularly express RANKL, unless bacterially challenged [13–15]. The potential effects of polymicrobial oral biofilm challenge have not been investigated in this respect. It is not known if supragingival and subgingival biofilms have different capacities in regulating the RANKL–OPG system. The present *in vitro* study aims to compare the capacity of a supragingival and a subgingival biofilm to regulate the expression of the RANKL–OPG system in human GF cultures. It is hypothesized that, due to its more virulent nature for the development of periodontitis, the subgingival biofilm would cause a more potent up-regulation of the RANKL/OPG expression ratio, potentially enhancing bone destruction.

2. Materials and methods

2.1. In vitro biofilm model

The 6-species suprgagingival Zürich biofilm model [16] used in this study consisted of *Veillonella dispar* ATCC 17748 (OMZ 493), *Fusobacterium nucleatum* KP-F8 (OMZ 598), *Streptococcus oralis* SK248 (OMZ 607), *Actinomyces naeslundii* (OMZ 745), *Streptococcus mutans* UAB159 (OMZ 918) and *Candida albicans* (OMZ 110).

The 10-species subgingival Zürich biofilm model [17] used in this study consisted of *Campylobacter rectus* (OMZ 697), *F. nucleatum* subsp. *vincentii* KP-F2 (OMZ 596), *Porphyromonas gingivalis* ATCC 33277T (OMZ 925), *Prevotella intermedia* ATCC 25611T (OMZ 278), *Tanerella forsythia* OMZ 1047, *Treponema denticola* ATCC 35405T (OMZ 661), *V. dispar* ATCC 17748T (OMZ 493), *A. naeslundii* OMZ 745, *S. intermedius* ATCC 27335 (OMZ 512), and *S. oralis* SK 248 (OMZ 607). Briefly, the supragingival or subgingival biofilms were grown in 24-well cell culture plates on sintered hydroxyapatite discs, resembling natural tooth surfaces, and were pre-conditioned for pellicle formation with human mixed saliva for 4 h. To initiate biofilm formation, hydroxyapatite discs were covered for 16.5 h with 1.6 ml of growth medium consisting of 60% saliva, 10% human serum (pooled from three donors), 30% FUM culture medium [18] and 200 μl of a bacterial cell suspension containing equal volumes and density from each strain. After 16.5 h of anaerobic incubation at 37 °C, the inoculum suspension was removed from the discs by 'dip-washing' using forceps, transferred into wells with fresh medium (60% saliva, 10% human serum, 30% FUM), and incubated for further 48 h in anaerobic atmosphere. During this time-period, the discs were dip-washed 3× and given fresh medium once daily. After a total 64.5 h of incubation, at an advanced stage of biofilm maturation, the culture supernatants were collected, filtered and stored at −80 °C. The composition of biofilms on the hydroxyapatite discs at the time of supernatant collection was performed by bacterial culture analysis, as previously described [16,17], and the respective bacterial counts are provided in Table 1. It is anticipated that supernatants from earlier stages of biofilm culture would be less virulent, or at least less representative an established biofilm stage. The bacterial protein concentration in these supernatants was determined by the BCA Protein Assay (Pierce). For the experiments, these biofilm supernatant preparations were diluted into the final cell culture medium and maintained in the cell culture for up to 24 h [17]. Their concentration is expressed as total protein (μg/ml) present in the cell cultures. While total bacterial protein was selected as a calibration measure to compare the effects of the two biofilm supernatants, qualitative analysis of individual proteins is less feasible due to their vast number in this complex mixture.

2.2. Cell cultures

Primary human GF cell lines were established as previously described [13,15]. Briefly, gingival tissue biopsies used were obtained from healthy young individuals, who had their first premolar removed during the course of orthodontic treatment. Ethical approval was granted by the Human Studies Ethical Committee of Umeå University, Sweden, and informed consent was given by the subject. The cells were passaged and cultured in Minimum Essential Medium Alpha (Gibco), supplemented with 5% heat-inactivated foetal bovine serum (Sigma), 50 U/ml penicillin, and 50 μg/ml streptomycin (Sigma). For the experiments, GF cells at passage 3 were seeded at concentration 10×10^3 cells/cm^2 in antibiotics-free and 5% FBS culture medium, and were allowed to attach for 24 h, maintaining a sub-confluent status. Thereafter, the cells were cultured for 6 or 24 h in the presence or absence of ascending protein concentrations of biofilm supernatants.

2.3. Cytotoxicity assay

Potential cytotoxic effects of the two biofilms on GF cultures were evaluated by measurement of the extracellularly released cytosolic lactate dehydrogenase (LDH), using the CytoTox96® Non-Radioactive Cytotoxicity Assay (Promega). The cultures were exposed to ascending biofilm protein concentrations up-to 300 μg/ml, for 6 h. The cell culture supernatants were collected, and the cell monolayer was lysed. The cell supernatants and lysates were centrifuged at 1000 rpm for 5 min and thereafter transferred into an optically clear 96-well plate (Petra-plastic, Switzerland), followed by addition of reaction solution and incubated for 30 min in the dark. The reaction was then stopped and the absorbance was measured at 490 nm in a BioRad 3550 microplate reader, subtracting background values from all samples.

2.4. RNA extraction and cDNA synthesis

After completion of the experiments, the culture supernatants were removed from the culture and the cell monolayers were washed twice in PBS before being lysed. The collected cell lysate was homogenized with QIAshredder (QIAGEN), and total RNA was extracted by using the RNeasy Mini Kit (QIAGEN), according to the manufacturer's instructions, and the RNA was finally eluted in 50 μl RNase free water and its concentration was measured by a NanoDrop spectrophotometer. One microgram of total RNA was then reverse transcribed into single-stranded cDNA by using M-MLV Reverse Transcriptase, Oligo(dT)$_{15}$ Primers, and PCR Nucleotide Mix according to the manufacturer's protocol (all from Promega), at 40 °C for 60 min, and 70 °C for 15 min. The resulting cDNA was stored at −20 °C until further use.

2.5. Quantitative real-time polymerase chain reaction (qPCR)

For RANKL and OPG gene expression analyzes, qPCR was performed in an ABI Prism 7000 Sequence Detection System and software (Applied Biosystems). 18S rRNA was used as endogenous RNA control in the samples (house-keeping gene). For the amplification reactions, the TaqMan Gene Expression Master Mix and Gene Expression Assay kits from Applied Biosystems were used

Download English Version:

https://daneshyari.com/en/article/2794673

Download Persian Version:

https://daneshyari.com/article/2794673

Daneshyari.com