Available online at www.sciencedirect.com

ScienceDirect

Biomedical Journal

journal homepage: www.elsevier.com/locate/bj

Original Article

Oral infection of mice with *Fusobacterium nucleatum* results in macrophage recruitment to the dental pulp and bone resorption

Larry Johnson [a,b], *Cássio Luiz Coutinho Almeida-da-Silva* [a,b], *Christina Maeda Takiya* [b], *Vanessa Figliuolo* [b], *Gustavo Miranda Rocha* [c], *Gilberto Weissmüller* [c], *Julio Scharfstein* [b], *Robson Coutinho-Silva* [b,1], *David M. Ojcius* [a,b,*,1]

[a] *Department of Biomedical Sciences, University of the Pacific, Arthur Dugoni School of Dentistry, San Francisco, CA, USA*

[b] *Immunobiology Program, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*

[c] *Molecular and Structural Biology Program, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*

ARTICLE INFO

Article history:
Received 24 December 2017
Accepted 8 May 2018
Available online 2 July 2018

Keywords:
Immunology
Inflammation
Periodontal disease
Dental
Innate immunity

ABSTRACT

Background: *Fusobacterium nucleatum* is a Gram-negative anaerobic bacterium associated with periodontal disease. Some oral bacteria, like *Porphyromonas gingivalis*, evade the host immune response by inhibiting inflammation. On the other hand, *F. nucleatum* triggers inflammasome activation and release of danger-associated molecular patterns (DAMPs) in infected gingival epithelial cells.

Methods: In this study, we characterized the pro-inflammatory response to *F. nucleatum* oral infection in BALB/c mice. Western blots and ELISA were used to measure cytokine and DAMP (HMGB1) levels in the oral cavity after infection. Histology and flow cytometry were used to observe recruitment of immune cells to infected tissue and pathology.

Results: Our results show increased expression and production of pro-inflammatory cytokines during infection. Furthermore, we observe that *F. nucleatum* infection leads to recruitment of macrophages in different tissues of the oral cavity. Infection also contributes to osteoclast recruitment, which could be involved in the observed bone resorption.

Conclusions: Overall, our findings suggest that *F. nucleatum* infection rapidly induces inflammation, release of DAMPs, and macrophage infiltration in gingival tissues and suggest that osteoclasts may drive bone resorption at early stages of the inflammatory process.

* *Corresponding author*. Department of Biomedical Sciences, University of the Pacific, Arthur Dugoni School of Dentistry, 155 Fifth St., San Francisco, CA 94103, USA.
E-mail address: dojcius@pacific.edu (D.M. Ojcius).

Peer review under responsibility of Chang Gung University.

[1] These authors share senior co-authorship.

https://doi.org/10.1016/j.bj.2018.05.001

At a glance commentary

Scientific background on the subject

The effect of *Fusobacterium nucleatum* on the immune response remains poorly understood in mouse models of oral infection.

What this study adds to the field

This study showed that oral infection with *F. nucleatum* stimulates inflammation and infiltration of macrophages in gingival tissue, which could lead to bone loss in the oral cavity.

The oral cavity is colonized with hundreds of different species of bacteria which compose the oral microbiome [1,2]. Some common bacteria found in individuals afflicted with periodontitis include *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, and *Aggregatibacter actinomycetemcomitans* [3,4]. Gingivitis is diagnosed when the gingiva, or gums, reveals signs of swelling, redness, or chronic bleeding [5], usually associated with gingival infection. However, chronic inflammation can lead to development of periodontitis with signs of deep periodontal pockets, alveolar bone resorption, and tooth loss [6,7].

The tooth is surrounded by the gingival epithelium. This microenvironment is optimal for growth of anaerobic bacteria and provides an opportunity for pathogenic bacteria to attach and coaggregate into biofilms [8]. *F. nucleatum* is one of the predominant bacteria and contributors to biofilm formation [9–11]. The bacteria utilize adhesion mechanisms of lectin-like and non-lectin-like interactions and adhesion peptides, such as FadA (*Fusobacterium* adhesin A) for attachment [12–16]. These interactions facilitate coaggregation or infiltration into lymphocytes, polymorphonuclear neutrophils, erythrocytes, epithelial cells, and fibroblasts [11,12,14,17,18].

The oral epithelium defends against bacterial colonization by secretion of antimicrobial peptides called defensins [4,19–21]. β-defensins target bacteria as the peptides are electrostatically attracted to their negative charged membranes and induce pore formation [4,19,22]. Antimicrobial peptides can also act as chemoattractants and recruit other immune cells, neutrophils or T cells [4,7,19]. Thus, β-defensins play an active role as part of innate and adaptive responses to oral infection.

Gingival epithelial cells (GECs) represent a major barrier to infection by invasive bacteria, and also contribute to immune recognition of the pathogens and the immune response [23,24]. When pathogen-associated molecular patterns (PAMPs) of bacteria are recognized by host pathogen recognition receptors (PRRs) on GECs, they activate NF-κB and induce expression of cytokines and chemokines, and recruit neutrophils and macrophages [25–27]. Recognition of *F. nucleatum* and *A. actinomycetemcomitans* infection results in production of cytokines such as IL-1β [28–30]. TNF-α and IL-17 can also synergize with IL-1β to enhance expression and production of other cytokines (e.g., IL-6) and defensins, along with endothelial activation to enhance the immune response [31–36].

Although the goal of inflammation is to resolve oral infection, it can also lead to bone resorption. Alveolar bone is one of the most dynamic bones in the body, as osteoclasts and osteoblasts continually induce bone remodeling to maintain homeostasis [37–40]. Osteoclasts are resorptive cells that are activated and differentiated by macrophage-colony stimulating factor, receptor activator of nuclear factor kappa-B ligand (RANKL)-RANK signaling, interleukins, and TNF-α [38,39,41–43]. Once osteoclasts adhere to bone, a ruffled border is created between the activated osteoclast and bone [38,40], and osteoclasts are able to degrade the mineral matrix [38,40]. Degraded bone matrix is removed as it is transcytosed in vesicles through osteoclasts, and fuses with cytoplasmic vesicles containing tartrate-resistant acid phosphatase (TRAP) to be released in the extracellular matrix [38,44]. Phagocytes remove the debris and osteoblasts are recruited for bone formation after osteoclasts detach from the bone [38].

F. nucleatum mechanisms for invasion and host response have been evaluated both *in vitro* and *in vivo* [3,45–48]. We have previously reported that *F. nucleatum* infection induces inflammasome activation and release of cytokines and danger signals in human GECs *in vitro* [29,46]. In this study, we examined the immune response to *F. nucleatum* oral infection in BALB/c mice, which had not been previously characterized.

Materials and methods

Bacteria

F. nucleatum (ATCC 25586) was cultured at 37 °C under anaerobic conditions in brain-heart infusion broth supplemented with yeast extract (5 mg/mL), hemin (5 μg/mL), and menadione (1 μg/mL). Erythromycin (5 μg/ml) was used as a selective agent for *F. nucleatum* as previously described [49]. After 24 h of growth, bacteria were collected by centrifugation at 6000 × *g* for 10 min at 4 °C, washed twice and resuspended with phosphate-buffered saline (PBS). Quantification of bacteria was measured by optical density (OD) to obtain a concentration of 10^9 colony-forming units (CFU)/ml using a reference standard.

Mice and oral challenge with F. nucleatum

BALB/c mice were obtained from the animal facility of the Institute of Biophysics Carlos Chagas Filho at the Federal University of Rio de Janeiro. All protocols used in this study followed the guidelines and were approved by the Institutional Animal Care and Use Committee at the Federal University of Rio de Janeiro (CEUA-UFRJ 076/15).

Six-to eight-week-old male BALB/c mice were given ad libitum water containing 10 mL of Bactrim (Roche) comprised of sulfamethoxazole/trimethoprim for 10 days. Then antibiotic-free water was given to the mice for 3 days prior to infection. The protocol for oral infection was adapted from Baker et al. [50]. On days of infection, mice were anesthetized with 100 μl of ketamine-xylazine solution (100 mg/ml and 20 mg/ml) by intraperitoneal injection. Anesthetized mice