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Lactoferrin inhibits infection-related osteoclastogenesis without interrupting compressive force-related osteoclastogenesis

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ABSTRACT

Background: Control of periodontal tissue inflammation during orthodontic treatment is very important in achieving a favourable therapeutic goal. We previously demonstrated that orally applied bovine lactoferrin (bLF) inhibited LPS-induced bone resorption but not orthodontic force-induced tooth movement in vivo. This study is designed to examine the underlying mechanism of it.

Methods: We examined the inhibitory effects of bLF on the expression of RANKL, OPG, TNF-α and COX-2 in osteoblasts loaded with compressive stress (CS) in comparison with LPS stimulated osteoblasts. Formation of osteoclasts was evaluated by co-culture system.

Results: Both CS- and LPS-applications upregulated COX-2 and RANKL but downregulated OPG. TNF-α was upregulated in LPS-stimulated osteoblasts but downregulated in CS-loaded osteoblasts. NS398 (a specific inhibitor of COX-2) significantly inhibited CS-induced RANKL-upregulation but not LPS-induced RANKL upregulation, indicating a critical role of COX-2/PGE_2 pathway in CS-induced osteoclastogenesis. bLF significantly downregulated LPS-induced upregulation of RANKL and eliminated OPG suppression but not affected in CS-induced changes. Moreover, bLF significantly decreased LPS-induced osteoclast formation, whereas bLF had no effect on PGE_2-induced osteoclast formation.

Conclusions: bLF can effectively suppress harmful bone destruction associated with periodontitis without inhibiting bone remodelling by CS-loading. Therefore, oral administration of bLF may be highly beneficial for control of periodontitis in orthodontic patients.

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1. Introduction

In orthodontic treatment, fixed appliances make oral hygiene difficult, and most of the orthodontic patients have some degree of gingival inflammation. In particular, there are risks of gingival recession, attachment loss, bone resorption and root resorption in the application of orthodontic force to the tooth with periodontal inflammation.[1] Therefore, adjunctive maintenance programmes for controlling periodontal inflammation that patients can do by themselves are necessary.[2]

Bovine lactoferrin (bLF); an iron-binding glycoprotein of transferrin family, is widely used as food additive and supplement with high safety.[3] It is well accepted that bLF has various biological activities including anti-viral,[4] anti-bacterial,[5] immunomodulatory and anti-inflammatory functions.[6–8] In various diseases such as colon cancer, hepatitis, and arthritis, oral administration of bLF has been used as an adjunctive strategy. Recently, we focused on the anti-inflammatory effect of bLF and examined the effects of oral administration of bLF on LPS-induced periodontal destruction in rat. Orally applied bLF inhibited LPS-induced osteoclastic bone resorption through suppression of TNF-α production from constitutive cells in periodontal tissues.[9] Moreover, 4 weeks of bLF application to periodontitis subjects resulted in an obvious improvement of probing depth and a significant decrease of LPS-induced cytokines production from peripheral blood mononuclear cells.[10] Notably, we recently reported that bLF attenuates LPS-induced bone resorption through reduction of RANKL and TNF-α production in osteoblasts and that bLF inhibits LPS and IL-1β induced osteoclastogenesis through a novel mechanism.[11] These findings indicate that oral administration of bLF may be used as an effective strategy for control of periodontal inflammation.

During orthodontic tooth movement, orthodontic force induces osteoclast formation on the compressive side which is a very essential process for tooth movement. Interestingly, we have recently demonstrated that orally applied bLF inhibited LPS-induced osteoclastogenesis in alveolar crestal area but not orthodontic force-induced osteoclastogenesis and tooth movement.[12] It strongly indicated that the oral administration of bLF may be used as an effective tool for control of periodontal inflammation in orthodontic patients. However, the underlying mechanisms of how bLF inhibits LPS-induced osteoclastogenesis without interrupting tooth movement remain unclear. In the present study, we investigated the bLF effects on compressive stress (CS)-induced osteoclastogenesis in comparison with those on LPS-induced osteoclastogenesis via osteoblasts in vitro.

2. Materials and methods

2.1. Reagents

bLF was purchased from Milk Industry (Morinaga Co. Ltd., Tokyo, Japan). LPS from *Aggregatibactor Actinomycetemcomitance* (ATCC29522 strain) (*A.a.*-LPS) was kindly provided by Professor Tatsuji Nishihara of Kyushu Dental College.

2.2. Cell line and cell culture

ST2 (a bone-marrow-derived osteogenic cell line) cells were maintained in a minimum essential medium alpha medium (α-MEM) (Invitrogen, Grand Island, USA) with 10 mM HEPES (pH 7.2), 10% FBS (Invitrogen) and 100 U/ml penicillin–streptomycin (Invitrogen) at 37 °C in a humidified atmosphere of 5% CO_2.

2.3. Gene expression experiments

2.3.1. Effects of bLF on gene expression of osteoclastogenesis-related cytokines in ST2 cells with LPS-stimulation or CS-application

ST2 cells were seeded in 6-well tissue culture plates (3.5×10^5 cells/well) and cultured in α-MEM containing 10% FBS for 2 days. Then, the cells were incubated with *A.a.*-LPS (100 ng/ml) or CS (0.5 g/cm^2) with or without 4 h-pretreatment with bLF (10 μg/ml). CS-application was performed according to the Kanazaki's methods.[13] Briefly, a glass cylinder was placed over a confluent cell layer in the well of a 6-well plate. The compressive force was adjusted by adding lead granules to the cylinder. The cultured ST2 cells were harvested for 2 h and 24 h after LPS-stimulation or CS-application.

2.3.2. Investigation of COX-2/PGE_2 pathway on osteoclastogenesis in ST2 cells with LPS-stimulation or CS-application

ST2 cells were seeded in 6-well tissue culture plates (3.5×10^5 cells/well) and cultured in α-MEM containing 10% FBS for 2 days. Then, the cells were incubated with *A.a.*-LPS (100 ng/ml) or CS (0.5 g/cm^2) with 2 h-pretreatment with NS398 (5.0 μM) (Cayman Chemical Company, Michigan, USA), which is a specific COX-2 inhibitor. The cultured ST2 cells were harvested for 24 h after LPS-stimulation or CS-application.

2.3.3. RNA extraction and RT-PCR analysis

Total RNA was extracted from the harvested cells using RNeasy Mini kit (Qiagen, Hilden, Germany). A measure of 1 μg of total RNA was used for cDNAs synthesis using Rever Tra Ace (TOYOBO, Osaka, Japan) according to the manufacturer's instruction.

Quantitative real-time RT-PCR was performed in the Real-Time PCR system (Applied Biosystems, Tokyo, Japan) using TagMan Fast Universal PCR Master Mix (Applied Biosystems) for TagMan assay, Fast SYBR Green Master Mix (Applied Biosystems) for SYBR Green assay. Reaction product was quantified with 18S as the reference gene. The specific PCR primers for TNF-α, COX-2, RANKL, OPG and 18S were presented in Table 1.

2.4. Cell preparation and osteoclast formation assay

Primary OBs were obtained from calvariae of newborn ddY mice by conventional methods using collagenase. Bone marrow cells (BMCs) were collected from the femora and