Available online at www.sciencedirect.com
SCIENCE DIRECT
Microbes and Infection 8 (2006) 1025–1034
Microbes and Infection
www.elsevier.com/locate/micinf

Original article

Proinflammatory gene expression in mouse ST2 cell line in response to infection by *Porphyromonas gingivalis*

Takashi Ohno [a,b], Nobuo Okahashi [a,*], Shinji Kawai [a], Takahiro Kato [a], Hiroaki Inaba [a], Yasuko Shibata [c], Ichijiro Morisaki [b], Yoshimitsu Abiko [c], Atsuo Amano [a]

[a] *Department of Oral Frontier Biology, Osaka University Graduate School of Dentistry, 1-8 Yamadaoka, Suita-Osaka 565-0871, Japan*
[b] *Division of Special Care Dentistry, Osaka University Graduate School of Dentistry, Suita-Osaka 565-0871, Japan*
[c] *Department of Biochemistry, Nihon University School of Dentistry at Matsudo, Chiba 271-8587, Japan*

Received 19 April 2005; accepted 23 October 2005
Available online 18 January 2006

Abstract

Porphyromonas gingivalis is a predominant periodontal pathogen, whose infection causes inflammatory responses in periodontal tissue and alveolar bone resorption. Various virulence factors of this pathogen modulate host innate immune responses. It has been reported that gingipains degrade a wide variety of host cell proteins, and fimbriae are involved in bacterial adhesion to and invasion of host cells. In the present study, we profiled ST2 stromal cell gene expression following infection with the viable *P. gingivalis* strain ATCC33277 as well as with its gingipain- and fimbriae-deficient mutants, using microarray technology and quantitative real-time polymerase chain reaction. Using a mouse array of about 20,000 genes, we found that infection with the wild strain elicited a significant upregulation (greater than 2-fold) of expression of about 360 genes in ST2 cells, which included the chemokines CCL2, CCL5, and CXCL10, and other proinflammatory proteins such as interleukin-6 (IL-6) and matrix metalloproteinase-13 (MMP-13). Further, infection with the gingipain-deficient mutant elicited a reduced expression of the CXCL10, IL-6 and MMP-13 genes, suggesting that gingipains play an important role in inducing the expression of those genes following *P. gingivalis* infection. On the other hand, the pattern of global gene expression induced by the fimbriae-deficient mutant was similar to that by the wild strain. These results suggest that *P. gingivalis* infection induces gene expression of a wide variety of proinflammatory proteins in stromal cells/osteoblasts, and gingipains may be involved in inducing several of the proinflammatory factors.

Keywords: *Porphyromonas gingivalis*; Inflammation; Osteoblast; Stromal cell; Microarray; Gene expression; Periodontitis

1. Introduction

Periodontitis is an inflammatory disorder of the supporting tissues of the teeth and one of the most common types of human infection. Alveolar bone resorption followed by loss of teeth is the most important clinical issue associated with the disease. A small subset of periodontal bacteria are supposed to play a major part in its pathogenesis, and *Porphyromonas gingivalis* is considered to be related to severe forms of adult periodontal diseases [1–3]. *P. gingivalis* is a Gram-negative black pigmented anaerobe that colonizes in periodontal pockets and then spreads into deeper tissues including connective and bone tissues [1–4]. Chronic inflammation following *P. gingivalis* colonization leads to the destruction of periodontal tissues, resorption of alveolar bone, and exfoliation of teeth [2,3,5–7]. The current investigations on bone diseases such as rheumatoid arthritis and periodontal disease revealed that inflammation is a key step in promoting bone destruction [8,9]. Many studies showed that osteoblasts and bone marrow-derived stromal cells are highly responsible to the development of local inflammatory responses, the interaction with

* Corresponding author. Tel./fax: +81 6 6879 2976.
E-mail address: okahashi@dent.osaka-u.ac.jp (N. Okahashi).

1286-4579/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.micinf.2005.10.021

osteoclast precursor cells, and the promotion of the osteoclast differentiation [8,9].

P. gingivalis produces various cellular components that have been proposed to function as virulence factors, including lipopolysaccharide (LPS) and fimbriae as well as cysteine-proteinases termed arginine (Arg)- and lysine (Lys)-gingipains [1,2,5–7,10,11]. These virulence factors easily diffuse and infiltrate into deeper periodontal tissues, resulting the destruction of the infected tissues [2,6,7,10]. LPS is a major component of the outer membrane of Gram-negative bacteria that displays multiple biological and immunological activities through mammalian innate receptors named toll-like receptors (TLRs) [12,13], and it has been reported that *P. gingivalis* LPS is a potent stimulator of inflammatory mediators such as interleukin-1 (IL-1) and prostaglandin E_2, which eventually induce bone resorption [1,6,14–16]. Fimbriae are reported to mediate the bacterial adherence to and invasion of epithelial cells and gingival fibroblasts [2,5,7,17–20]. Gingipains degrade collagen and fibronectin, and inhibit the interaction between host cells and the extracellular matrix. Gingipains also degrade various cytokines, resulting in a disturbance of the host cytokine network [10,11,21]. Although LPS of *P. gingivalis* is supposed to play the most critical role in inducing proinflammatory responses in infected periodontal tissues, several investigators have suggested that fimbriae-dependent invasion of this pathogen triggers the production of proinflammatory mediators in human endothelial and epithelial cells [22–24]. In addition, we and others have found that gingipains were involved in the production of several proinflammatory cytokines such as IL-6 from host cells [25,26]. Therefore, these bacterial factors contribute differentially in the progression of the overall inflammatory destruction of the infected periodontal tissues.

Interactions between the pathogen and host cells leads to the activation of gene expression; however, the genetic profiles of infected cells differ among different microorganisms [27–31]. Recent technological advances have provided several widely used methods for large-scale studies of gene expression, including gene macro- and microarrays, and oligonucleotide chips. DNA microarrays have become powerful tools to study host-microbe interactions, and investigations using such microarrays have been performed to analyze the transcriptional pattern of host genes during infection of epithelial cells with *Salmonella* and *Shigella* [27,30].

In the present study, we used microarray systems to explore global cellular gene expression profiles following *P. gingivalis* infection of ST2 stromal cell line with some pre-osteoblastic characters. Further, the expression levels of specifically selected genes associated with inflammatory responses were determined by real-time reverse transcriptase–polymerase chain reaction (RT–PCR) assays. In order to investigate the role of fimbriae and gigipains in inducing proinflammatory responses in host cells, fimbriae- and gingipains-deficient mutants of this pathogen were also employed to the microarray and real-time PCR studies. Our results revealed that stromal cells/osteoblasts produce an array of immune regulatory molecules in response to *P. gingivalis* infection, recruiting leukocytes to the sites of bacterial infection. In addition to the bacterial LPS, gingipains may be involved in inducing these proinflammatory responses.

2. Materials and methods

2.1. Bacterial strains and LPS

Porphyromonas gingivalis ATCC33277, KDP136 (a triple deficient mutant for three gingipains, Arg-gingipain-A, -B and Lys-gingipain of ATCC33277) [21], and KDP150 (a fimbriae-deficient mutant of ATCC33277) [32] were grown in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, MD) supplemented with yeast extract (0.1%; BBL), hemin (5 μg/ml; Sigma Chemicals, St. Louis, MO) and menadione (1 μg/ml; Sigma), as described previously [17,21]. Bacterial cells were harvested by centrifugation, then washed with phosphate-buffered saline (PBS), and resuspended in alpha minimum essential medium (αMEM; Gibco-BRL, Grand Island, NY) containing no antibiotics. The numbers of bacteria were determined with a spectrophotometer (at an optical density at 600 nm) based on a standard curve established by colony formation on bacterial plates [17]. *P. gingivalis* LPS was extracted from lyophilized cells of *P. gingivalis* ATCC33277 by the hot phenol–water procedure. The crude extract was treated with nuclease and washed extensively with pyrogen-free water by ultracentrifugation. The chemical and immunobiological properties of this LPS have been previously reported [14].

2.2. Cell culture and RNA preparation

A mouse ST2 cell line (bone marrow derived stromal cell line) was obtained from Riken Bioresource Center (Tsukuba, Japan), and used in this study. The cells were grown in an incubator at 37 ° C, with 5% CO_2, in αMEM supplemented with 10% heat-inactivated fetal calf serum (FCS) (Gibco-BRL), penicillin G (100 U/ml), and streptomycin (100 μg/ml). In order to avoid non-specific biological effects of growth factors or hormones contained in the FCS, infection of cells with *P. gingivalis* was performed in serum-free medium containing no antibiotics. The ST2 cells were washed three times with serum-free αMEM containing no antibiotics, subjected to infection with viable *P. gingivalis* at a multiplicity of infection (MOI) of 10, 50 or 100, and cultured for 1, 3, or 6 h in an incubator at 37 ° C, with 5% CO_2. Following infection, the cells were washed with PBS, and total RNA was extracted using an RNeasy Mini kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions. Integrity and purity of the RNA samples were analyzed with an Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA).

2.3. CodeLink microarray

A CodeLink mouse 20 K bioarray system (Amersham Biosciences, Buckinghamshire, UK), which contains a collection of approximately 20,000 probes of 30 bases in length, was used to explore the global cellular gene expression profiles. RNA samples were reverse transcribed followed by in vitro