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Catechins inhibit CXCL10 production from oncostatin M-stimulated human gingival fibroblasts

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Abstract

CXC chemokine ligand 10 (CXCL10) plays a pivotal role in the recruitment of Th1 cells and, thus, in the development of periodontal disease. Epigallocatechin gallate (EGCG) and epicatechin gallate (ECG), the major catechins derived from green tea, have multiple beneficial effects, but the effects of catechins on CXCL10 production from human gingival fibroblasts (HGFs) is not known. In this study, we investigated the mechanisms by which EGCG and ECG inhibit oncostatin M (OSM)-induced CXCL10 production in HGFs. HGFs constitutively expressed glycoprotein 130 and OSM receptor beta (OSMRβ), which are OSM receptors. OSM increased CXCL10 production in a concentration-dependent manner. EGCG and ECG prevented OSM-mediated CXCL10 production by HGFs. Inhibitors of p38 mitogen-activated protein kinase, c-Jun N-terminal kinase (JNK), phosphatidylinositol-3-OH kinase and signal transducer and activator of transcription (STAT)3 decreased OSM-induced CXCL10 production. EGCG significantly prevented OSM-induced phosphorylation of JNK, Akt (Ser473) and STAT3 (Tyr705 and Ser727). ECG prevented phosphorylation of JNK and Akt (Ser473). In addition, EGCG and ECG attenuated OSMRβ expression on HGFs. These data provide a novel mechanism through which the green tea flavonoids, catechins, can provide direct benefits in periodontal disease.

Keywords: Catechins; Oncostatin M; CXCL10; Human gingival fibroblasts

1. Introduction

Oncostatin M (OSM) belongs to the interleukin (IL)-6 family of cytokines, which includes IL-6, leukemia-inhibitory factor, IL-11, cardiotrophin-1 and ciliary neurotrophic factor [1]. The IL-6 family of cytokine receptors requires dimerization with glycoprotein 130 (gp130), a glycoprotein cell surface receptor, for intracellular signaling. OSM and several members of the IL-6 cytokine family are known to activate fibroblasts and to regulate the synthesis of matrix metalloproteinases and their inhibitors in these cells [2–4]. However, the results of studies in humans, rats and mice support the notion that OSM is uniquely involved in the regulation of inflammation [5–7]. OSM is primarily produced in and released by activated monocytes, T lymphocytes and neutrophils [8–10], and it is found in a variety of inflammatory sites. In the human lung during acute lung injury, infiltrating neutrophils secrete OSM [5]. OSM levels also are elevated in the sera of patients with rheumatoid arthritis [11] as well as in patients with inflamed skin [12] and periodontitis [13]. In addition, in vitro studies have demonstrated that OSM not only regulates the remodeling function of fibroblasts but also elicits inflammatory responses in these cells. OSM induces the CC chemokines eotaxin [7] (an eosinophil chemoattractant) and monocyte chemoattractant protein 1 [3] in the mouse lung and synovial fibroblasts, respectively. Moreover, overexpression of OSM in the mouse lung results in increased recruitment of eosinophils [7]. Taken together, these studies suggest that OSM regulates inflammatory function in fibroblasts and that fibroblasts may be implicated in the recruitment of leukocytes upon activation by OSM.

Periodontitis is a chronic bacterial infection of tooth-supporting structures. It causes destruction of periodontal connective tissues and bone. The disease initiation and progression result from the host response to plaque bacteria. Immunohistochemical studies have revealed dense inflammatory cell infiltration, including T and B cells and macrophages in periodontitis-affected regions [14–19]. Recently, several studies demonstrated that Th1 cells are involved in bone resorption in oral cavities. Kawai et al. [20] reported that the activation of Th1-type T cells appeared to trigger inflammatory periodontal bone resorption. Stashenko et al. [21] reported that intrapulpal challenge with viable *Porphyromonas gingivalis* results in massive periapical bone destruction during systemic Th1 response. However, the effects of OSM on the Th1 response in periodontal disease are uncertain.

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Gingival fibroblasts, the major cell type in periodontal connective tissues, provide a tissue framework for tooth anchorage. Until recently, they were presumed to be immunologically inert. Currently, however, researchers recognize their active role in host defense. Upon stimulation with cytokines as well as with bacterial pathogens, human gingival fibroblasts (HGFs) secrete various soluble mediators of inflammation such as IL-1β, IL-6 and IL-8 [22–25] and up-regulate expression of HLA-DR, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 [26]. These fibroblast-derived mediators and surface antigens are thought to play an important role in the periodontal inflammatory response.

Catechins are naturally occurring polyphenolic compounds, which have been shown to have anti-inflammatory, antioxidant and free radical scavenging properties in vitro [27,28]. For example, epigallocatechin gallate (EGCG), one of the major isoforms of the catechins, has been shown to inhibit the infiltration of leukocytes and myeloperoxidase activity and to decrease UV-B-induced erythema [29]. Catechins have also been shown to decrease the production of the proinflammatory cytokines IL-1β and tumor necrosis factor (TNF)-α and to enhance the production of the anti-inflammatory cytokine IL-10 [30,31]. However, reports concerning the effects of catechins on chemokine production are rare.

CXC chemokine ligand 10 (CXCL10) was discovered as an IFN-γ-inducible protein of 10 kDa in the monocytic U937 cells [32]. CXCL10 attracts activated Th1 cells through interaction with CXC chemokine receptor 3 (CXCR3) [33,34]. CXCL10 shares this receptor and, hence, biological activity with two more recently identified CXC chemokines, CXCL9 and CXCL11 [35–37]. In vivo, enhanced levels of CXCL10 have been reported in several inflammatory diseases that are predominantly associated with a Th1 phenotype. It is reported that CXCL10 and CXCR3 are detected in inflamed gingival tissues [38,39]. However, it is unknown whether HGFs are related to CXCL10 production in inflamed gingival tissues.

The aim of this study was to examine the effect of OSM on CXCL10 production by HGFs. Moreover, we examined the effects of the catechins EGCG and epicatechin gallate (ECG) on CXCL10 production from OSM-stimulated HGFs. Furthermore, we investigated whether catechin treatment modified phosphorylation of mitogen-activated protein kinases (MAPK), Akt or signal transducer and activator of transcription (STAT)3 in OSM-stimulated HGFs.

2. Materials and methods

2.1. Gingival tissue biopsies and cell culture

We used HGFs isolated from three clinically healthy gingiva during routine distal wedge surgical procedures. Gingival specimens were cut into small pieces and transferred to culture dishes. The HGFs that grew from the gingivae were primarily cultured on 100-mm^2 uncoated plastic dishes in Dulbecco's modified Eagle's medium (Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA) and antibiotics (penicillin G, 100 U/ml; streptomycin, 100 μg/ml) at 37°C in humidified air with 5% CO_2. Confluent cells were transferred and cultured for use in the present study. After three to four subcultures by trypsinization, the cultures contained homogeneous, slim and spindle-shaped cells growing in characteristic swirls. The cells were used for experiments after five passages. Informed consent was obtained from all subjects participating in this study. The study was performed with the approval and compliance of the University of Tokushima Ethical Committee.

2.2. RNA extraction and reverse transcription–polymerase chain reaction (RT-PCR) analysis

Total RNA was prepared from gingival biopsies or HGF using the RNeasy Total RNA isolation kit (Qiagen, Hilden, Germany). Single-strand cDNA for a PCR template was synthesized from 48 ng of total RNA using the oligo$(dT)_{12-18}$ primer (Invitrogen, Carlsbad, CA, USA) and superscript3 reverse transcriptase (Invitrogen) under the conditions indicated by the manufacturer. Specific primers were designed from cDNA sequences for gp130, OSM receptor beta (OSMRβ) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Each cDNA was amplified by PCR using Hot Star *Taq* DNA polymerase (Qiagen). The sequences of the primers were as follows: gp130-F, 5′-CATGCTTTGGGTGGAATGGAC-3′; gp130-R, 5′-CATCAACAGGAAGTTGGTCCC-3′; OSMRβ-F, 5′-GTGTGGGTGCTTCTCCTGCTTCTGTA-3′; OSMRβ-R, 5′-TCTGTGCTAATGACTGTGCTTGTGGT-3′; GAPDH-F, 5′-TGAAGGTCGGAGTCAACGGATTTGGT-3′; GAPDH-R, 5′-CATGTGGGCCATGAGGTCCACCAC-3′. The conditions for PCR were as follows: 1× (95°C for 15 min), 35× (94°C for 1 min, 59°C for 1 min, 72°C for 1 min) and 1× (72°C for 10 min). The products were analyzed on a 1.5% agarose gel containing ethidium bromide. We did not detect any bands when we performed PCR without adding the cDNA template in this study.

2.3. Flow cytometric analyses

Following the required culture time, the cells were washed twice with ice-cold PBS. HGFs were harvested by incubation with PBS–4 mmol/L EDTA. Most of the cells were rounded up following this treatment and removed by gentle agitation. Any cells that failed to detach were removed with gentle scraping. The cells were washed twice with ice-cold PBS and incubated (20 min on ice) in PBS–1% bovine serum albumin (BSA). The cells were incubated with mouse anti-human gp130 antibody (R&D Systems, Minneapolis, MN, USA; 5 μg/ml), mouse anti-human OSMRβ antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA; 5 μg/ml) or an isotype control antibody on ice for 30 min. After being washed three times with PBS–1% BSA (Sigma), the cells were incubated with an FITC-conjugated rabbit anti-mouse F $(ab')_2$ fragment (DAKO, Kyoto, Japan) or FITC goat anti-rat IgG(H+L) Conjugate (ZYMED Laboratories, South San Francisco, CA, USA) for 30 min on ice. After being washed three times with PBS–1% BSA, the cells were immediately analyzed with flow cytometry (Epics XL-MCL; Coulter, Hialeah, FL).

2.4. CXCL10 production by HGFs

HGFs were stimulated with OSM (PeproTech, Rocky Hill, NJ, USA) for 24 h. The supernatants from HGFs were collected, and the CXCL10 concentrations of the culture supernatants were measured in triplicate with ELISA. Duoset (R&D Systems) was used for the determinations. All assays were performed according to the manufacturer's instructions, and cytokine levels were determined using the standard curve prepared for each assay. In selected experiments, HGFs were cultured for 1 h in the presence or

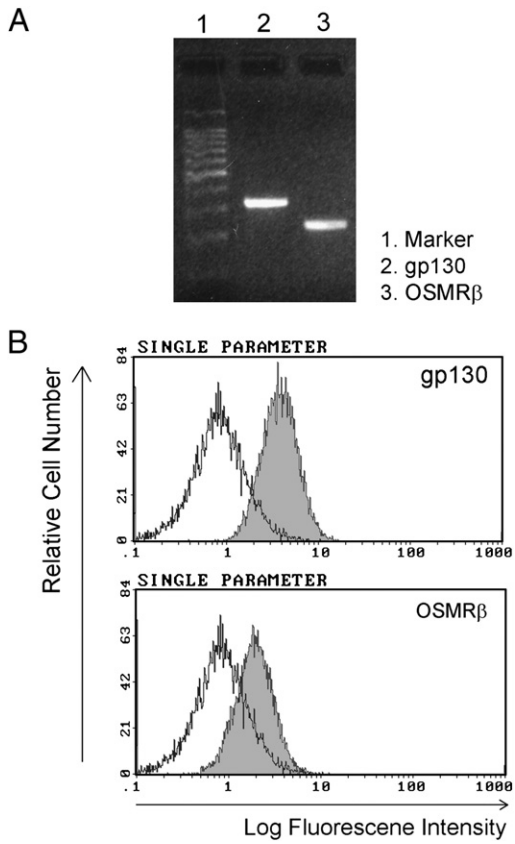

Fig. 1. gp130 and OSMRβ expression by HGFs. (A) Total RNA was prepared from nonstimulated HGFs. The expression of gp130 and OSMRβ mRNA in nonstimulated HGFs was analyzed by RT-PCR, as described in Materials and methods. (B) Flow cytometric analysis of gp130 and OSMRβ expression by nonstimulated HGF. The filled area represents gp130 or OSMRβ-specific fluorescence, and the empty area represents the background level of fluorescence caused by the secondary antibody.