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Research article

Mitochondrial reactive oxygen species mediate the lipopolysaccharide-induced pro-inflammatory response in human gingival fibroblasts

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ABSTRACT

Although periodontal diseases are initiated by bacteria that colonize the tooth surface and gingival sulcus, the host response is believed to play an essential role in the breakdown of connective tissue and bone. Mitochondrial reactive oxygen species (mtROS) have been proposed to regulate the activation of the inflammatory response by the innate immune system. However, the role of mtROS in modulating the response of human gingival fibroblasts (HGFs) to immune stimulation by lipopolysaccharides (LPS) has yet to be fully elucidated. Here, we showed that LPS from *Porphyromonas gingivalis* stimulated HGFs to increase mtROS production, which could be inhibited by treatment with a mitochondrial-targeted exogenous antioxidant (mito-TEMPO) or transfection with manganese superoxide dismutase (MnSOD). A time-course study revealed that an increase in the concentration of mtROS preceded the expression of inflammatory cytokines in HGFs. Mito-TEMPO treatment or MnSOD transfection also significantly prevented the LPS-induced increase of interleukin (IL)-1β, IL-6, and tumor necrosis factor-α. Furthermore, suppressing LPS-induced mtROS generation inhibited the activation of p38, c-Jun N-terminal kinase, and inhibitor of nuclear factor-κB kinase, as well as the nuclear localization of nuclear factor-κB. These results demonstrate that mtROS generation is a key signaling event in the LPS-induced pro-inflammatory response of HGFs.

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1. Introduction

Periodontitis consists of a group of infections leading to the inflammation of gingival tissues and the destruction of periodontal tissues [1]. Human gingival fibroblasts (HGFs) are a major constituent of gingival connective tissue. The ability of these periodontal residential cells to recognize pathogens makes them crucial in dealing with microbial invasion. These cells release several inflammatory cytokines including interleukins (ILs), and thus play active roles in host defense. However, HGFs from diseased sites contribute to the pathogenesis of periodontitis [2].

Lipopolysaccharides (LPS) from gram-negative bacteria are potent inducers of pro-inflammatory mediators and can initiate a number of host-mediated destructive processes [3]. HGFs stimulated by LPS produce pro-inflammatory cytokines such as IL-1β, IL-6, and tumor necrosis factor-α (TNF-α), which are important in the initiation of periodontitis [4]. Increased IL-1β, IL-6, and TNF-α secretion by fibroblasts has been detected in periodontitis lesions, and their unrestricted production may contribute to chronic leukocyte recruitment and tissue destruction [5]. Regulating the inflammatory response of HGFs is one way of preventing and/or controlling the progression of periodontitis [6].

Reactive oxygen species (ROS) have historically been viewed as toxic metabolic by-products and causal agents in a myriad of human pathologies. The first evidence for a role of ROS in the periodontal tissues was the demonstration that these molecules were produced by neutrophils and caused the deterioration of surrounding tissues [7]. However, recent work indicated that ROS were critical intermediaries in cellular signaling pathways. For

Abbreviations: DMEM, Dulbecco's Modified Eagle's Medium; ELISA, enzyme-linked immunosorbent assay; EMSA, electrophoretic mobility-shift assay; H_2DCFDA, 5,6-chloromethyl-2′,7′- dichlorodihydrofluorescein diacetate, acetyl ester; HGF, human gingival fibroblast; IKK, inhibitor of nuclear factor-κB kinase; IL, interleukin; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharides; MAPK, mitogen-activated protein kinase; MnSOD, manganese superoxide dismutase; mtROS, mitochondrial reactive oxygen species; NF, nuclear factor; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PDTC, pyrrolidine dithiocarbamate; qRT-PCR, quantitative reverse transcription polymerase chain reaction; ROS, reactive oxygen species; tMnSOD, transfected manganese superoxide dismutase; TNF, tumor necrosis factor

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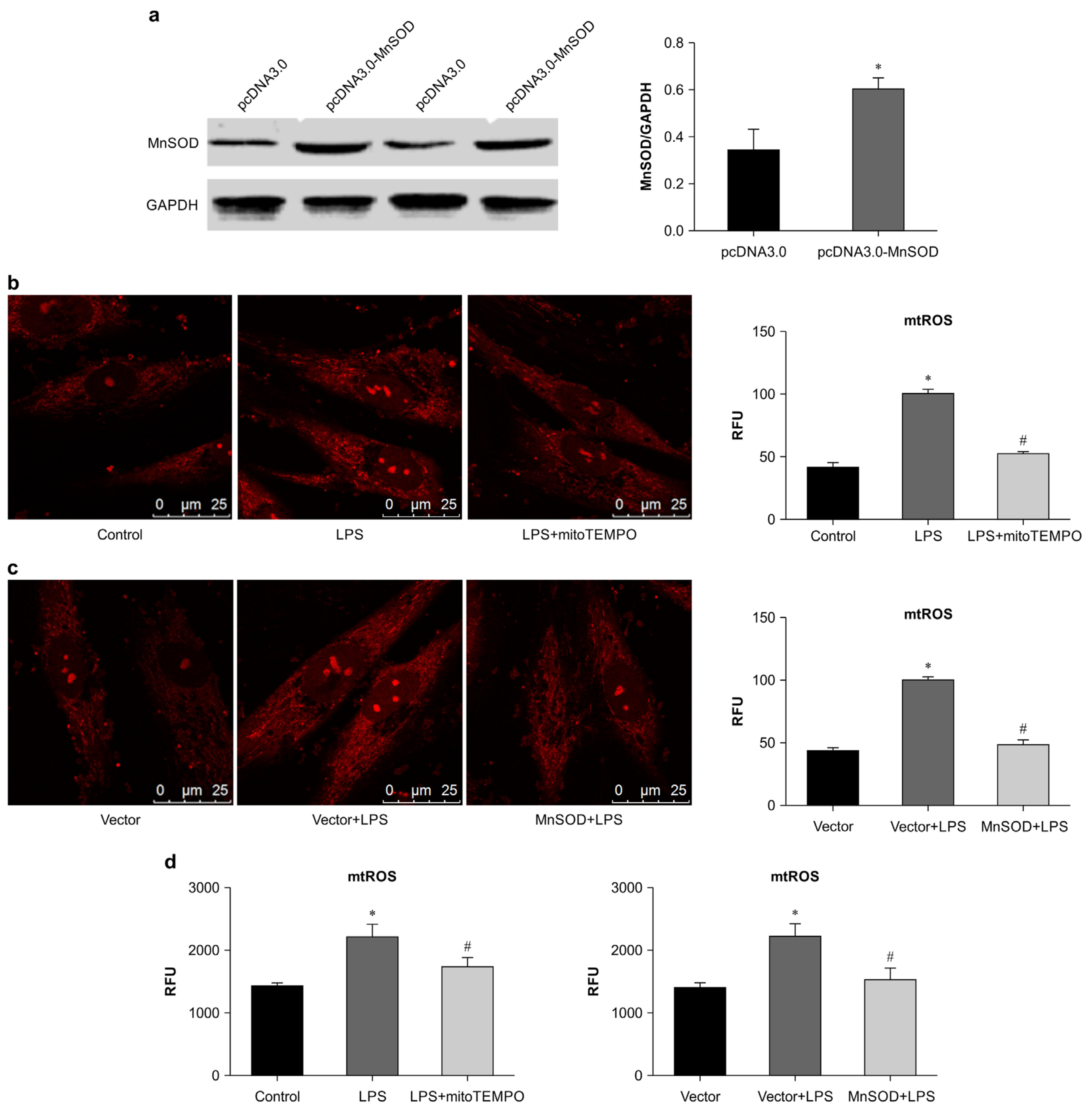

Fig. 1. Manganese superoxide dismutase (MnSOD) was overexpressed in human gingival fibroblasts (HGFs) by transfecting pcDNA3.0-MnSOD (tMnSOD) (a). Inhibitory effect of Mito-TEMPO or tMnSOD on the lipopolysaccharides (LPS)-stimulated augmentation of mtROS generation. HGFs were stimulated with LPS for 9 h in the absence or presence of the indicated concentration of Mito-TEMPO, or with the overexpression of MnSOD, and then incubated with a mitochondrial reactive oxygen species (mtROS) indicator, MitoSOX. Then, mtROS levels were analyzed by immunofluorescence microscopy (b, c) or using a multimode microplate reader (d). Data are presented as the mean ± standard deviation (SD) ($n=3$). $^{*}p<0.05$ (treated group compared to control group); $^{\#}$ $p<0.05$ (blocked group compared to treated group).

example, ROS generated in the periodontal tissues upon stimulation by LPS from *Porphyromonas gingivalis* (*P. gingivalis*) contributed to the pathogenesis of periodontal disease by activating matrix-degrading metalloproteinases and up-regulating pro-inflammatory cytokines [8]. In addition, ROS modified pro-inflammatory gene expression by altering kinase cascades and activating transcription factors, including mitogen-activated protein kinase (MAPK) and nuclear factor (NF)-κB [9–11].

Although mitochondria serve as the major intracellular source of ROS in most cells, few studies have examined the contribution of mitochondrial (mt)ROS to regulating the production of pro-inflammatory cytokines in HGFs stimulated by LPS. Recent publications have indicated that mtROS act as signaling molecules to trigger pro-inflammatory cytokines production [12–15]. In addition, Park et al. reported that the mitochondrion was the major source of LPS-stimulated ROS in microglial cells. Furthermore, they found that inhibiting ROS generation modulated the production of pro-inflammatory mediators by preventing LPS-induced MAPK and NF-κB activation [15].

These observations provide much-needed clarification regarding the cellular source of ROS that induces the production of certain pro-inflammatory cytokines. Based on these findings, it is possible that the suppression of mtROS might alleviate inflammation [15]. However, the role of mtROS production in HGFs has yet to be fully elucidated. Thus, in this study, we determined whether mtROS were associated with the generation of pro-

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