Molecular Immunology 77 (2016) 8–13

Contents lists available at ScienceDirect

Molecular Immunology

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

# Manipulation of necroptosis by *Porphyromonas gingivalis* in periodontitis development

Xiaojing Ke[1], Lang Lei[1], Huang Li, Houxuan Li*, Fuhua Yan*

*Nanjing Stomatological Hospital, Medical School of Nanjing University, China*

ARTICLE INFO

*Article history:*
Received 20 June 2016
Received in revised form 14 July 2016
Accepted 14 July 2016
Available online 21 July 2016

*Keywords:*
*Porphyromonas gingivalis*
Receptor-interacting protein
Necroptosis
Periodontitis

ABSTRACT

To eliminate invading pathogens and keep homeostasis, host employs multiple approaches such as the non-inflammation associated-apoptosis, inflammation associated-necroptosis and pyroptosis, etc. Necroptosis is known as a highly pro-inflammatory form of cell death due to the release of massive damage-associated molecular patterns (DAMPs). For the first time, we reported that *Porphyromonas gingivalis* induced cellular necroptosis through receptor-interacting protein 1 (RIP1)/RIP3/mixed lineage kinase domain-like (MLKL) signaling pathway in monocytes. Necroptosis in THP-1 cells was induced by MLKL phosphorylation in vitro. *P. gingivalis* treated-THP-1 cells exhibited lower cell death rate with pretreatment of inhibitors RIP1 and MLKL, accompanied with attenuated TNF-α and IL-6 expressions. Moreover, the necroptosis risk was also reduced via gene silencing by RIP3 or MLKL in the *P. gingivalis* treated-THP-1 cell lines. We further explored *P. gingivalis*-induced necroptosis in animal models in vivo. Firstly, C57BL/6 mice were injected with *P. gingivalis* in the subcutaneous chamber model. Animals pretreated with MLKL inhibitor exhibited significantly enhanced *P. gingivalis* clearance; in addition, levels of TNF-α and IL-6 were notably decreased by 60% via MLKL inhibition. Secondly, *P. gingivalis*-induced periodontitis was utilized to investigate necroptosis related-periodontopathogensis. Positive staining of phosphorylated MLKL in mice periodontitis biopsies was detected to a higher degree, while larger amount of alveolar bone loss was observed in MLKL (−) group comparing to those in the MLKL (+) group. These findings may suggest that *P. gingivalis* play essential roles in necroptosis process during periodontitis, and our research may shed light on the further work on the related periodontopathogenesis investigation.



## 1. Introduction

Chronic periodontitis is an oral inflammatory disease leading to the destruction of periodontium, and *Porphyromonas gingivalis*, a Gram-negative anaerobic bacterium, is implicated as a major putative periodontal pathogen (Pihlstrom et al., 2005). The invasive *P. gingivalis* acquires plenty of properties to invade periodontal tissues and avoid immune clearance, such as fimbrae to adhere to host cells, proteases, namely gingipains, to degrade extracellular matrix, and lipopolysacchride (LPS) to elicit a host of immune responses. While present at relatively low colonization levels, the community-wide dysbiotic effect of *P. gingivalis* has led to its characterization as a keystone pathogen, that is, an organism with a disproportionately large effect on its environment relative to its abundance (Hajishengallis et al., 2012; Zenobia and Hajishengallis, 2015). Upon *P. gingivalis* proliferation and infection, a dense infiltration of inflammatory cells, including monocytes and macrophages, occurs in the periodontal milieu. Activated inflammatory cells release a multitude of cytokines as a defense against bacterial invasion (Pathirana et al., 2000); however, excessive growth of periodontal pathogens and production of various pathogenic factors in the periodontal micro-environment pose a great challenge for the survival of periodontal host cells.

Previously, the death of host cells in the periodontal tissue was considered largely as an unregulated accidental cell death form, i.e. necrosis, while apoptosis was the only form of regulated cell death (Pasparakis and Vandenabeele, 2015). Apoptosis may contribute to pathogen clearance but do not elicit host inflammation; therefore, the host manipulates other pro-inflammatory forms of cell death to alert the danger signal during bacterial infection.

Indeed, new forms of regulated cell death have been uncovered in both infection and sterile inflammation (Vanden Berghe et al., 2014). For example, *P. gingivalis* can activate pyroptosis, a caspase-1 dependent form of cell death that generates IL-1β release and inflammatory responses (Park et al., 2014). Furthermore, necroptosis, another newly discovered highly pro-inflammatory mode of

* Corresponding authors at: Nanjing Stomatological Hospital, Medical School of Nanjing University, 30 Zhongyang Road, Nanjing, Jiangsu, 210008, China.
*E-mail addresses:* lihouxuan3435_0@163.com (H. Li), yanfh@nju.edu.cn (F. Yan).
[1] These authors contributed equally to the work.

http://dx.doi.org/10.1016/j.molimm.2016.07.010

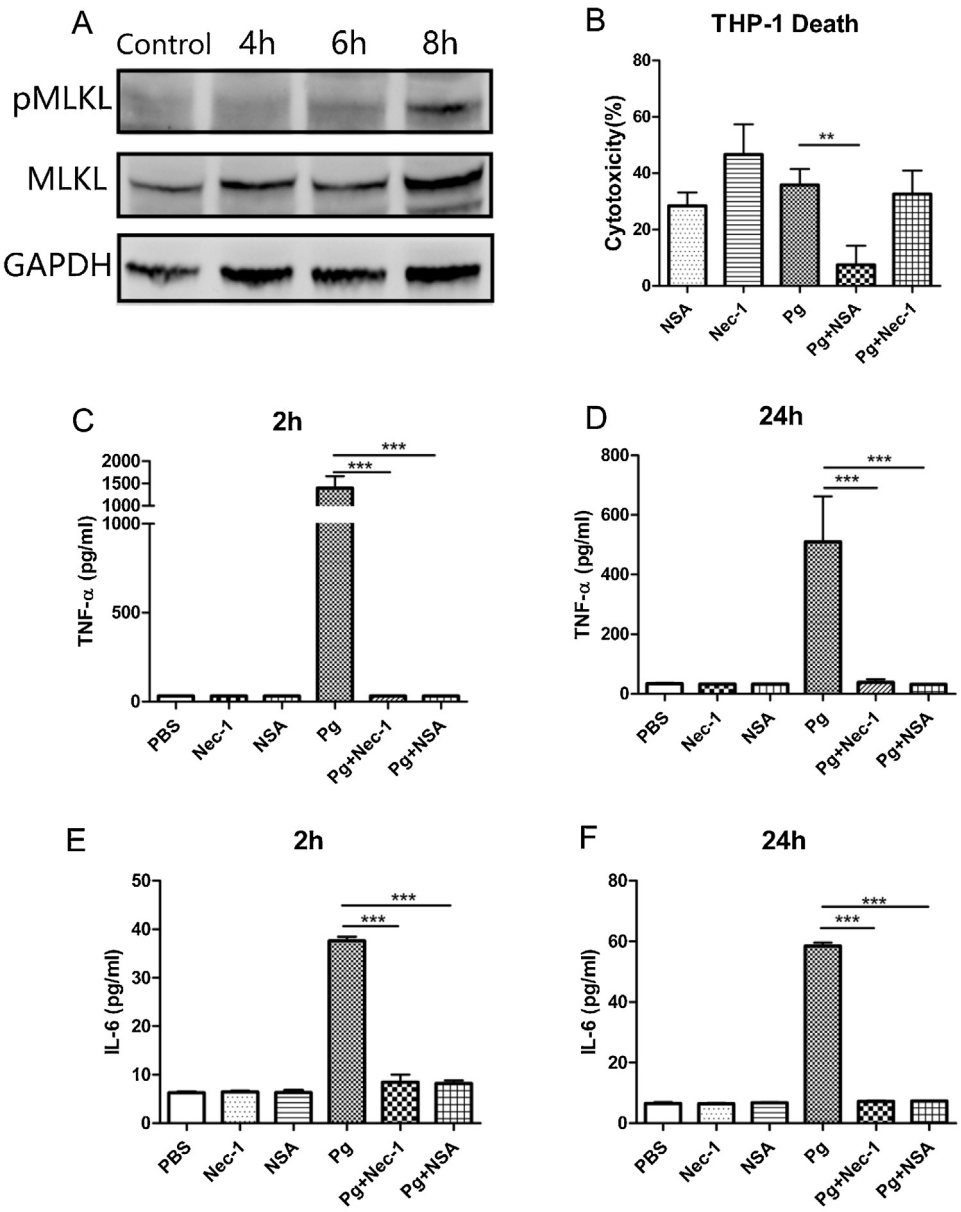


**Fig. 1.** *P. gingivalis* induced necroptosis in monocytes. (A) THP-1 cells were stimulated with *P. gingivalis* MOI100 and probed with antibodies against phosphorylated MLKL (pMLKL), MLKL and GAPDH. (B) THP-1 cells were pretreated for 2 h with necrostatin-1 (Nec-1) or necrosulfonamide (NSA) and incubated with *P. gingivalis* MOI 100 for 4 h; then cell death was determined by LDH assay. (C-F) Levels of TNF-α and IL-6 in supernatants were quantified by ELISA. (**, $p < 0.01$, ***, $p < 0.001$). Data were representative of two independent experiments with three technical replicates (mean and SD).

cell death (Kaczmarek et al., 2013; Welz et al., 2011), has been found to participate in inflammatory diseases. In the absence of caspase-8 activation, receptor-interacting serine-threonine kinase (RIP)1 and RIP3 interacts through the RIP homotypic interaction motifs (RHIMs) activating RIP3, which in turn phosphorylates mixed lineage kinase domain-like (MLKL) leading to MLKL pore formation and loss of plasma membrane integrity (Chen et al., 2014; Pasparakis and Vandenabeele, 2015). As a consequence, necroptosis leads to the release of intracellular contents and exposure of damage-associated molecular patterns (DAMPs) (Kaczmarek et al., 2013).

However, the role of necroptosis in the pathogenesis of periodontitis, which is primarily a bacteria-induced infection in the periodontal supporting structures, has never been explored. Here we for the first time demonstrated that *P. gingivalis* manipulated the TLR-RIP1/3-MLKL-mediated necroptosis pathway to generate a robust but detrimental inflammatory response in periodontal niche, and necroptosis may be blocked for therapeutic intervention during *P. gingivalis* induced periodontitis.

## 2. Materials and methods

### 2.1. Bacterial strains

*P. gingivalis* strain ATCC 33277 was grown anaerobically in modified BHI medium (contains 5 μg/ml hemin, 1 μg/ml menadione and 1 μg/ml yeast extract) at 37 °C with 85% $N_2$, 5%$H_2$ and 10% $CO_2$overnight to obtain live bacteria in exponential growth. Using a spectrophotometer, the bacterial concentrations were standardized to an optical density of 1 at 600 nm, which corresponds to $10^9$ CFU/ml.