International Immunopharmacology 38 (2016) 214–222

Contents lists available at ScienceDirect

International Immunopharmacology

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

Pseudomonas aeruginosa promotes autophagy to suppress macrophage-mediated bacterial eradication

Yongjian Wu [a,b,1], Dandan Li [d,1], Yi Wang [a,b,1], Kang Chen [a,b,e], Kun Yang [a,b], Xi Huang [a,b], Yuanqing Zhang [c,*], Minhao Wu [a,b,**]

[a] Department of Immunology, Institute of Tuberculosis Control, Institute of Human Virology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510080, China
[b] Key Laboratory of Tropical Diseases Control (Sun Yat-sen University), Ministry of Education, Guangzhou 510080, China
[c] School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China
[d] Department of Clinical Laboratory, Jinling Hospital, School of Medicine, Nanjing University, Nanjing 243000, China
[e] Division of Clinical Laboratory, Zhongshan Hospital of Sun Yat-sen University, Zhongshan 528403, China

ARTICLE INFO

Article history:
Received 24 February 2016
Received in revised form 12 April 2016
Accepted 29 April 2016
Available online xxxx

Keywords:
Autophagy
Phagocytosis
Bacterial killing
Macrophages
Pseudomonas aeruginosa

ABSTRACT

Objectives: To explore the role of autophagy on macrophage-mediated phagocytosis and intracellular killing of *Pseudomonas aeruginosa* (PA), a common extracellular bacterium which often causes various opportunistic infections.
Methods: Macrophages were infected with PA or stimulated with zymosan bioparticles. Autophagy was tested by fluorescent microscopy and Western blot for LC3. Phagocytosis and killing efficiency were assessed by plate count assay, flow cytometry or immunofluorescent staining. Phagocytic receptor expression, ROS generation and NO production were examined by PCR, flow cytometry and Griess reaction, respectively.
Results: PA infection induced autophagy activation in both mouse and human macrophages. Induction of autophagy by rapamycin or starvation significantly inhibited PA internalization by downregulating phagocytosis receptor expression, and suppressed intracellular killing of PA via reducing ROS and NO production in macrophages. While knockdown of autophagy molecules ATG7 or Beclin1 enhanced macrophage-mediated phagocytosis and intracellular killing of PA. Additionally, confocal microscopy data showed that induction of autophagy reduced the number of phagosomes and phagolysosomes in macrophages after stimulation with zymosan bioparticles.
Conclusions: Our study suggested that PA promotes autophagy to suppress macrophage-mediated bacterial phagocytosis and intracellular killing. These insights demonstrated a novel immune evasion mechanism employed by PA, which may provide potential therapeutic strategies of PA infectious diseases.

1. Introduction

Pseudomonas aeruginosa (PA) is a Gram-negative extracellular bacterium, which commonly exists in the environment and causes various opportunistic infectious diseases [1], such as keratitis in contact lens users [2], and nosocomial infections in patients with burning wounds, cystic fibrosis [3], or immunodeficiency [1]. Pathogenesis of these diseases largely results from bacterial virulence factors, such as exoenzyme ExoU, endotoxin lipopolysacharide (LPS) and exotoxin, which cause host cell death and tissue damage [4,5]. In recent decades, host antimicrobial immunity attracts more and more attention, because of the big challenge of drug-resistance in traditional antibiotic therapies [6].

Mononeuclear phagocyte system (MPS) is one of the most important components in the host immune system [7]. MPS system consists of the phagocytic cells located in reticular connective tissue, including monocytes, macrophages, as well as specialized macrophages like Kupffer cells, Langerhans cells, microglia and osteoclasts [7]. These cells recognize invading pathogens via distinct pattern recognition receptors expressed on the cell surface [8–10], and initiate the innate immune defense response. And engulf invading pathogens with the help of several phagocytic receptors on the cell surface [11], such as scavenger receptor (SR) [12], mannose receptor (MR) [13], Fc receptors for IgG (FcγR) [14], complement receptor (CR) [15], etc. After that, activated phagocytes produce a large amount of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [16,17], to kill the engulfed bacteria.

In addition to phagocytosis and intracellular killing, autophagy is another evolutionarily conserved cellular process in the innate immunity [18]. Autophagy promotes degradation of cytosolic components such as damaged organelles, misfolded proteins, and intracellular microorganisms via a lysosome-dependent pathway [19,20]. Initiation of autophagy is usually characterized by the formation of microtubule-

* Correspondence to: Y. Zhang, Sun Yat-sen University School of Pharmaceutical Sciences, 132 Waihuan East Road, Guangzhou 510006, China.
** Correspondence to: M. Wu, Sun Yat-sen University, Zhongshan School of Medicine, 74 Zhongshan 2nd Road, Guangzhou 510080, China.
E-mail addresses: zyqsinap@gmail.com (Y. Zhang), wuminhao@mail.sysu.edu.cn (M. Wu).
[1] The authors contribute equally to this article.

http://dx.doi.org/10.1016/j.intimp.2016.04.044
1567-5769/

Table 1
Nucleotide sequence of the specific primers used in PCR amplification.

Gene	Primer sequence (5′-3′)	
Human CR	AGCCCCTAGATGTACAGTGA	F
	AGACCTGCCTTTTAATCGGA	R
Mouse CR	GTCCTCAGCAGGAGAGTGACA	F
	ACTCGTCCGAGTACTGCATCA	R
Human SR	CTGCTCCGAATCTGTGAAAT	F
	GATGAGAACTGCAAACACGA	R
Mouse SR	AGGCTCTGCCCTCATGAACA	F
	GCAGCCTTGAAGGACTTCAACT	R
Human MR	TGCTACTGAACCCCCACAA	F
	AGAGGAACCCATTCGAAGACA	R
Mouse MR	TGAGCAACCACTAGGTTACA	F
	TTGCATCAGTGAAGGTGGA	R
Human FcγR	TCCATCCCACAAGCAAACCA	F
	GCAATGACCACAGCCACAAT	R
Mouse FcγR	TCAACGGAACAGCCGTTCA	F
	CCTGGAGTAGCAGCCAATCA	R
Human β-actin	GCT CCT CCT GAG CGC AAG	F
	CAT CTG CTG GAA GGT GGA CA	R
Mouse β-actin	GATTACTGCTCTGGCTCCTAGC	F
	GACTCATCGTACTCCTGCTTGC	R
Mouse iNOS	CCACATCTGGCAGAATGAGAA	F
	TGAAGGCGTAGCTGAACAA	R

associated protein light chain 3 (LC3) puncta, as well as the increased amount of LC3-II which is closely correlated with the number of autophagosomes [21]. Then autophagosomes are fused with lysosomes to form autophagolysosomes, where the cytosolic proteins or pathogens undergo degradation [19,20]. Autophagy is critical in regulating cellular homeostasis and host antimicrobial immunity [22,23]. However, the mechanism responsible for the induction and regulation of autophagy are poorly understood. Studies have demonstrated that autophagy can be induced in response to various stress conditions, such as starvation, rapamycin, TLR ligands, inflammatory cytokines (e.g., IFN-γ and TNF) as well as bacterial infection [24]. Both autophagy and phagocytosis pathways are stimulated by pathogen associated molecular patterns (PAMPs) [24,25] and have critical roles in pathogen capture and degradation [25]. However, the interaction between autophagy and phagocytosis remains controversial [26].

In our study, we demonstrated that PA infection induced autophagy in both RAW264.7 cells and THP-1 macrophages. However, induction of autophagy inhibited phagocytosis and intracellular killing of PA in macrophages, by decreasing expression of phagocytic receptors and ROS/NO production, respectively. While knockdown of ATG7 or Beclin1 enhanced macrophagy-mediated phagocytosis and intracellular killing of PA. These findings increased our understanding of the interactions of autophagy, phagocytosis and killing, which may provide potential targets of therapeutic treatment of PA infectious diseases.

2. Materials and methods

2.1. Materials and reagents

PA strain 19,660 was purchased from the American Type Culture Collection (ATCC, Manassas, VA). Pseudomonas isolation agar was purchased from BD Difco Laboratories (Sparks, MD). Primary antibody

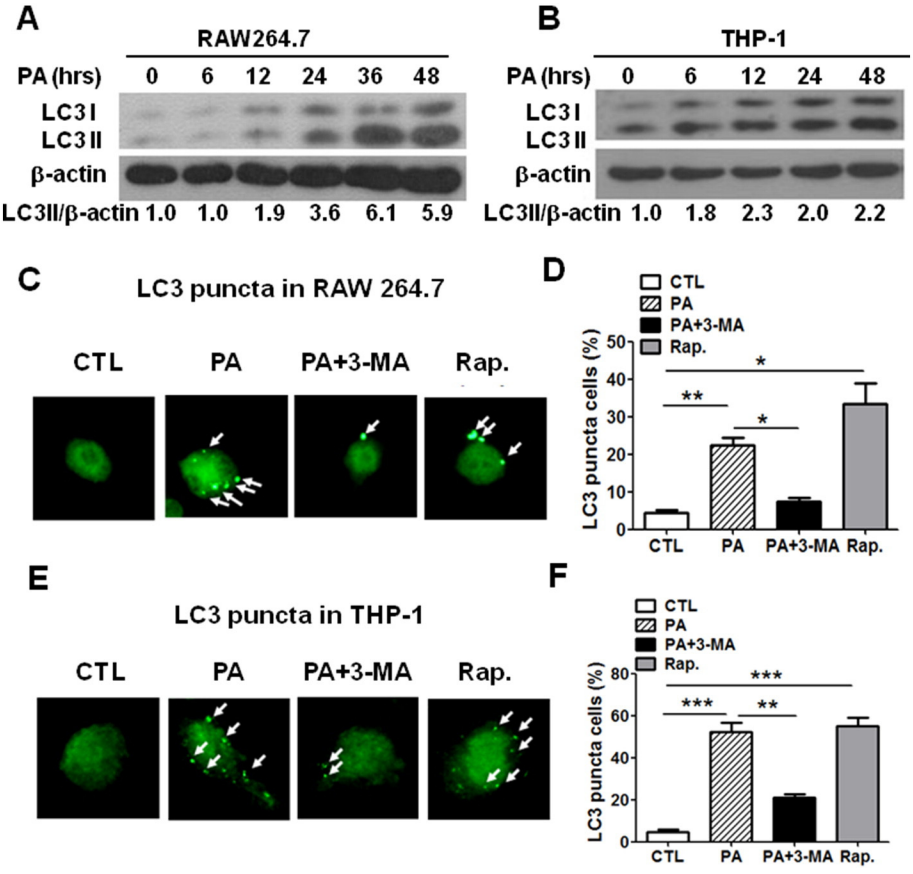

Fig. 1. PA infection induced autophagy in macrophages. (A–B) Protein levels of LC3-II were tested by Western-blot in RAW264.7 cells (A) and THP-1 macrophages (B) before or after PA infection for indicated times. Data shown represent three independent experiments. (C and E) RAW264.7 cells (C) and THP-1 macrophages (E) were pretreated with or without 3-methyladenine (3-MA, 10 μM, 2 h), followed with PA infection at MOI 25 for 1 h. Cells treated with rapamycin (4 μM, 6 h) were used as positive control of autophagy induction. LC3 puncta were detected by immunefluorescence microscopy. Arrows indicate the LC3 puncta. (D and F) Quantification of cells containing LC3 puncta in RAW264.7 cells (D) and THP-1 macrophages (F). Data are shown as the mean ± SEM of three independent experiments. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.