Microbes and Infection 16 (2014) 553–561

www.elsevier.com/locate/micinf

Original article

A *Salmonella enterica* conjugative plasmid impairs autophagic flux in infected macrophages

Yuanyuan Chu[1], Yanru Yang[1], Yuanyuan Li, Ying Ye, Jing Yan, Ting Wang, Shuyan Wu*, Rui Huang*

Department of Microbiology, Medical College of Soochow University, No. 199, Ren Ai Road, Suzhou, Jiangsu 215123, PR China

Received 20 November 2013; accepted 7 May 2014
Available online 22 May 2014

Abstract

pR_{ST98} was originally isolated from *Salmonella enterica* serovar typhi and could be transferred among enteric bacilli by conjugation. Our previous studies indicated that it could intervene in autophagy of host cells, while the mechanism remained undefined. Here, we explored how pR_{ST98} influenced the autophagic flux of murine macrophage-like cell line (J774A.1). *S. enterica* serovar typhimurium wild type strain (χ3306), harboring a 100 kb virulence plasmid, was used as a positive control. pR_{ST98} was transferred into χ3306 virulence plasmid cured strain (χ3337) to create the transconjugant strain (χ3337/pR_{ST98}). The bacterial strains incubated with J774A.1 revealed that survival rate of intracellular bacteria carrying pR_{ST98} was higher than that of plasmid free strain; presence of pR_{ST98} decreased the number of autophagy vacuoles, LC3 positive and p62 positive bacteria, and also the level of LC3-II and degradation of p62 in macrophages. After intervention with autophagy inhibitor chloroquine, the amount of LC3-II and autophagy vacuoles were still lower in macrophages infected with strains carrying pR_{ST98}. Our study suggested that pR_{ST98} could block or delay the formation of autophagosome in the earlier autophagy process, but couldn't affect the function of autolysosome. This finding provided novel insights into the role of enteric conjugation plasmid in bacterial pathogenesis.

Keywords: *Salmonella*; Plasmid; Macrophage; Chloroquine; Autophagic flux

1. Introduction

Salmonella enterica serovar typhi (*Salmonella typhi*), a gram-negative facultative foodborne pathogen, usually causes a broad spectrum of diseases in human such as food poisoning, gastroenteritis, typhoid fever and other intestinal infectious diseases, and it remains a global health problem. *S. typhi* infections are serious disease, especially in low-income countries, causing about 16 million cases with at least 600 thousand deaths annually worldwide [1]. In order to colonize host successfully, *S. typhi* has evolved a variety of strategies to overcome immune defense system of immuno-competent adults, and persistently maintains their own survival in human [2,3]. Previously, we found that strains of *S. typhi* in our laboratory, isolated from the blood of patients, harbored a 98.6 mDa plasmid designated as pR_{ST98}. Typhoid fever caused by *S. typhi* carrying pR_{ST98} results in severe illness status, more complications and high mortality [4]. Moreover, pR_{ST98} could be transferred among *S. typhi*, *S. enterica* serovar typhimurium (*Salmonella typhimurium*), *Escherichia coli* (*E. coli*) and *Shigella flexneri* in laboratory. Therefore, understanding the mechanisms that how enteric conjugation plasmid evades or subverts host defense to survive and establish a persistent infection is important for therapeutic intervention in infectious diseases of *S. typhi*. However, *S. typhi*, a human obligatory pathogen, is normally unable to infect mice, and this limits the study of *S. typhi* plasmid pR_{ST98}. Unlike *S. typhi*, *S. typhimurium* has a broad host range, causing diseases in both animals and human. Therefore, we transformed pR_{ST98} which was a conjugative

* Corresponding authors. Tel.: +86 512 65880132; fax: +86 512 65880103.
E-mail addresses: shuyanzw@aliyun.com (S. Wu), hruisdm@163.com (R. Huang).
[1] Yuanyuan Chu and Yanru Yang contributed equally to this work.

http://dx.doi.org/10.1016/j.micinf.2014.05.001

plasmid to *S. typhimurium* for research of its function in vivo and in vitro.

Autophagy is an intracellular innate defense pathway in response to a variety of stress stimuli [5,6]. Several lines of evidence have indicated that autophagy acts as a mechanism for eliminating intracellular bacteria through lysosomal degradation, a process referred to as xenophagy [7,8]. Recent reports have previously investigated the *S. typhimurium*-macrophage interaction in vitro, and found that macrophage autophagy plays a major role in response to *Salmonella* invasion [9,10]. As a selective pathway, xenophagy of *Salmonella* is based on the recognition and degradation of the bacteria, which depends on receptor proteins, including p62/SQSTM1, optineurin and NDP52, simultaneously binding autophagosome scaffold LC3 and bacteria [11]. Monitoring autophagic flux is a more reliable indicator to assess and differentiate the induction and suppression of autophagy. In the presence and absence of starvation and a lysosomotropic reagent of chloroquine (CQ), it was illuminated by measuring autophagic substrate degradation (herein referred to bacteria, p62 and LC3) in macrophages infected with *S. typhimurium*. Accumulating evidence indicates that bacteria modify the fate to resist host defense by targeting certain autophagy pathway, such as *Listeria monocytogenes*, can actively survive in host cells and exploit host defense for persistent infection by regulation of LC3 recruitment to bacteria [12]. *Salmonella*-induced autophagy is typically a dynamic, rapid and localized response; thus *Salmonella* infection is considered to be a useful model to study the relationship between autophagy process and the outcome of infectious diseases [13,14]. Monitoring autophagic flux would illuminate whether the conjugative plasmid pR_{ST98} of *S. enterica* may somehow interact with macrophage autophagy.

Our laboratory has previously investigated the plasmid pRST98, carrying the genes encoding properties of drug resistance and virulence [4]. *S. typhimurium* harbouring pRST98 may have been conferred novel strategies for intervene macrophage autophagy in order to survive and multiply inside J774A.1 cells. Studies of our laboratory indicated that pR_{ST98} exhibited significant cytotoxicity through modulating autophagy and apoptosis in host immune cell during the infection process of *Salmonella* [15–17]. Since autophagy involves dynamic and complicated processes, treatment of CQ is often used to analyze the block of autophagy pathway in different stage. In the present study we showed, for the first time by monitoring autophagic flux, the conjugative plasmid pR_{ST98} of *Salmonella* targeted and blocked autophagy pathway at the early stage of autophagosome formation. This finding provided novel insights into the role of enteric conjugation plasmid in bacterial pathogenesis.

2. Materials and methods

2.1. Bacterial strains and culture conditions

S. typhimurium strains χ3306 and χ3337 were kindly provided by Professor Roy Curtiss III [18]. All bacterial strains used in this study are listed in Table 1. All strains were grown to mid-logarithmic phase at 37 °C in Luria–Bertani (LB) broth, and quantified spectrophotometrically by determining OD 600 nm. Then they were centrifuged at 3000 × *g* for 10 min and resuspended in RPMI 1640 medium before adding to cells.

2.2. Mutant strains construction

Transconjugant strain χ3337/pR_{ST98} was constructed by a conjugal transfer of pR_{ST98} to χ3337. According to reference [15], pR_{ST98} was transferred from *S. typhi* wild type to plasmid-free *E. coli* K12W1485Rif^r F$^-$ Lac$^+$ (*E. coli* K12W1485); using *Shigella* and *Salmonella* selective plates containing rifampicin (100 μg/ml) and chloramphenicol (20 μg/ml), we could acquire *E. coli* K12W1485/pR_{ST98}. And then pR_{ST98} was similarly transferred from *E. coli* K12W1485/pR_{ST98} to χ3337. Specifically, overnight cultures of the donor and recipient bacteria were prepared in LB broth separately, and then took 0.1 ml of each culture to mix together and incubated for 4 h at 37 °C. The mixture were centrifuged at 2300× *g* for 5 min and resuspended in normal saline, then transferred to a LB plate and grown at 37 °C overnight. The collected lawn was diluted and spread on selective plates described above. The colonies producing hydrogen sulfide

Table 1
Bacterial strains used in the study.

Strain	Relevant genotype	Description (reference[s])
Serovar Typhi strains		
S. typhi-WT	*spv*$^+$, $pR_{ST98}$$^+$	Chimeric plasmid pR_{ST98} positive, Tc^r Amp^r Cm^r Sm^r Kn^r Cb^r [4]
Serovar Typhimurium strains		
χ3306	*spv*$^+$, pStSR$^+$	Virulence plasmid pStSR positive, Nal^r [18]
χ3337	*spv*$^-$, pStSR$^-$	Virulence plasmid-cured derivative of χ3306, Nal^r [18]
χ3337/pR_{ST98}	*spv*$^+$, $pR_{ST98}$$^+$ pStSR$^-$	pR_{ST98} was transferred into χ3337 by conjugation, Tc^r Amp^r Cm^r Sm^r Kn^r Cb^r Nal^r [This study]
χ3306*lux*	*spv*$^+$, pStSR$^+$ *lux*	Electroporational transformation of *lux* gene into χ3306, Nal^r [This study]
χ3337*lux*	*spv*$^-$, pStSR$^-$ *lux*	Electroporational transformation of *lux* gene into χ3337, Nal^r [This study]
χ3337/pR_{ST98}*lux*	*spv*$^+$, $pR_{ST98}$$^+$ *lux*, pStSR$^-$	Electroporational transformation of *lux* gene into χ3337/pR_{ST98}, Tc^r Amp^r Cm^r Sm^r Kn^r Cb^r Nal^r [This study]
χ3306-*rfp*	*spv*$^+$, pStSR$^+$ RFP	$CaCl_2$ transformation of pGMDs3 into χ3306, Amp^r Nal^r [This study]
χ3337-*rfp*	*spv*$^-$, pStSR$^-$ RFP	$CaCl_2$ transformation of pGMDs3 into χ3337, Amp^r Nal^r [This study]
χ3337/pR_{ST98}-*rfp*	*spv*$^+$, $pR_{ST98}$$^+$ RFP, pStSR$^-$	$CaCl_2$ transformation of pGMDs3 into χ3337/pR_{ST98}, Tc^r Amp^r Cm^r Sm^r Kn^r Cb^r Nal^r [This study]

r represents resistance.