Virology 446 (2013) 199–206

Contents lists available at ScienceDirect

Virology

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

Interleukin-1 receptor-associated kinase M (IRAK-M) promotes human rhinovirus infection in lung epithelial cells via the autophagic pathway

Qun Wu [a], Linda F. van Dyk [b,c], Di Jiang [a], Azzeddine Dakhama [d], Liwu Li [e], Steven R. White [f], Ashley Gross [a], Hong Wei Chu [a,c,*]

[a] Department of Medicine, National Jewish Health, Denver, CO, USA
[b] Department of Microbiology, University of Colorado Denver School of Medicine, Aurora, CO, USA
[c] Department of Immunology, University of Colorado Denver School of Medicine, Aurora, CO, USA
[d] Department of Pediatrics, National Jewish Health, Denver, CO, USA
[e] Department of Biological Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA
[f] Department of Medicine, University of Chicago School of Medicine, Chicago, IL, USA

ARTICLE INFO

Article history:
Received 9 April 2013
Returned to author for revisions
20 May 2013
Accepted 6 August 2013
Available online 30 August 2013

Keywords:
Human rhinovirus
IRAK-M
Viral replication
Autophagy
Airway epithelial cells

ABSTRACT

Human rhinovirus (HRV) is the most common viral etiology in acute exacerbations of asthma. However, the exact mechanisms underlying HRV infection in allergic airways are poorly understood. IL-13 increases interleukin-1 receptor associated kinase M (IRAK-M) and subsequently inhibits airway innate immunity against bacteria. However, the role of IRAK-M in lung HRV infection remains unclear. Here, we provide the first evidence that IRAK-M over-expression promotes lung epithelial HRV-16 replication and autophagy, but inhibits HRV-16-induced IFN-β and IFN-λ1 expression. Inhibiting autophagy reduces HRV-16 replication. Exogenous IFN-β and IFN-λ1 inhibit autophagy and HRV-16 replication. Our data indicate the enhancing effect of IRAK-M on epithelial HRV-16 infection, which is partly through the autophagic pathway. Impaired anti-viral interferon production may serve as a direct or an indirect (e.g., autophagy) mechanism of enhanced HRV-16 infection by IRAK-M over-expression. Targeting autophagic pathway or administrating anti-viral interferons may prevent or attenuate viral (e.g., HRV-16) infections in allergic airways.

Introduction

Human rhinovirus (HRV) is the most common viral etiology in acute exacerbations of chronic respiratory diseases such as asthma, chronic obstructive pulmonary diseases, and cystic fibrosis (Contoli et al., 2009; Kim and Gern, 2012; Tan, 2005; Wat et al., 2008). HRV infection often leads to more severe and longer duration of lower respiratory tract symptoms in patients than in healthy individuals, significantly increases healthcare costs and negatively impacts the quality of life of patients and their families (van Elden et al., 2008). Previous studies have shown that experimental infection with HRV-16 (a major group of HRV) in allergic asthma patients causes airway hyperresponsiveness compared with normal control subjects (Bardin et al., 1994; Fraenkel et al., 1995; Gern et al., 1997; Gern and Busse, 1999). However, the exact mechanisms underlying HRV-induced disease exacerbations are poorly understood.

Airway epithelial cells represent the primary site of respiratory microbial infections including HRV (Mosser et al., 2005; Papadopoulos et al., 2000). The degree of HRV replication in epithelial cells strongly influences the severity of respiratory symptoms during HRV infection. However, how host factors affect the course of HRV infection in airway epithelial cells remains unclear. In this study, we seek to explore the role of interleukin-1 receptor associated kinase M (IRAK-M) in HRV infection. IRAK-M, also known as IRAK-3, negatively regulates toll-like receptor (TLR)-mediated inflammatory responses to bacteria (Kobayashi et al., 2002; Rosati and Martin, 2002). We previously demonstrated epithelial IRAK-M up-regulation in asthmatic airways. Moreover, we found that IL-13, a Th2 cytokine prominent in asthma, increases airway epithelial IRAK-M and subsequently inhibits innate immunity against bacterial infections (Wu et al., 2012). Although recent studies have shown that hepatitis C virus and influenza virus up-regulate IRAK-M in human antigen presenting cells and in mice (Chung et al., 2010; Seki et al., 2010), IRAK-M function in viral infections has not been investigated. Virus-induced production of type I (e.g., IFN-β) and III (e.g., IFN-λ1)

Abbreviations: ALI culture, Air-liquid interface culture; HRV, Human rhinovirus; IRAK-M, Interleukin-1 receptor associated kinase M; IFN-β, Interferon-β; IFN-λ1, Interferon-λ1; PE, phosphatidylethanolamine

* Correspondence to: Department of Medicine, National Jewish Health, 1400 Jackson Street, Room A639, Denver, CO 80206, USA. Fax: +1 303 270 2319.
E-mail address: chuhw@njhealth.org (H.W. Chu).

0042-6822/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2013.08.005

interferons provides a critical first-line host defense against viruses (Sykes et al., 2012; Vareille et al., 2012). Deficiency of HRV-induced IFN-β and IFN-λ1 has been reported in asthmatic lungs, including bronchial epithelial cells (Baraldo et al., 2012; Contoli et al., 2006, Sykes et al., 2012; Wark et al., 2005). However, whether IRAK-M up-regulation in asthmatic airway epithelium regulates anti-viral responses is unknown.

Autophagy is an essential homeostatic pathway by which cells degrade damaged or obsolete organelles and proteins through the lysosomal machinery (Glick et al., 2010; Xie and Klionsky, 2007). Recent studies suggest autophagy is a novel mechanism by which the host defends against viral infection. Notably, autophagy recently has shown to exert both anti- and pro-viral functions. A recent study in HeLa cells demonstrated that autophagy promotes replication of HRV-2 (a minor group of HRV) (Klein and Jackson, 2011). Likewise, poliovirus exploits the autophagy machinery to favor its replication (Jackson et al., 2005; Taylor et al., 2009; Taylor and Jackson, 2009). Interestingly, poliovirus and HRV are both RNA viruses within the family of *Picornaviridae*. So far, the role of authophagy in the major group of HRV (e.g., HRV-16) infection and its regulation by IRAK-M has not been elucidated.

Central to the autophagy biogenesis is the formation of a double membrane-bound vesicle known as autophagosome, which undergoes several microtubule-dependent maturation events, including fusion with endosomes, multilamellar vesicles and lysosomes (Cesen et al., 2012; Mizushima et al., 2002). Beclin 1 plays an essential role in the initiation of autophagy (Cao and Klionsky, 2007; Liang et al., 1999). This process is mainly through promoting the activity of class III phosphoinositide 3-kinase (PI 3-kinase) Vps34 (Zeng et al., 2006). LC3 is pivotal to the formation of autophagosomes and has two forms: LC3 I and LC3 II (Kabeya et al., 2000). LC3 II is generated by the conjugation of LC3 I with phosphatidylethanolamine (PE) and is localized in the autophagosome membrane. Conversion of LC3 I into LC3 II is considered as a marker of autophagy (Klionsky et al., 2012; Tang et al., 2013).

In this study, we hypothesized that IRAK-M promotes lung HRV-16 infection via the autophagic pathway. To test our hypothesis, we first examined if IRAK-M over-expression in human lung epithelial cells promotes HRV-16 infection. Second, we knocked down beclin 1 in lung epithelial cells by using RNA interference to confirm the role of autophagy in IRAK-M-mediated HRV-16 replication. Third, we investigated whether IRAK-M promotes HRV-16 replication through inhibiting IFN-β and IFN-λ1 production in lung epithelial cells. Lastly, we determined the direct effects of exogenous IFN-β and IFN-λ1 on lung epithelial autophagy and HRV-16 infection.

Results

IRAK-M over-expression promotes HRV-16 replication

The IRAK-M over-expressing human lung epithelial NCI-H292 cell line (IRAK-M OE) and empty vector NCI-H292 cell line (EV) were established as previously described (Wu et al., 2012). HRV-16 dose response and time course studies were performed in IRAK-M OE and EV NCI-H292 cells with various doses of HRV-16 (3×10^2, 10^3, 3×10^3 and 10^4 $TCID_{50}$/well) or sterile phosphate-buffered saline (PBS) for 4 and 24 h to examine IRAK-M expression, viral RNA levels, and viral particles in cell supernatants. At the time of HRV infection, both cell lines reached the similar confluence (about 80%). No significant difference in cell growth (density) was observed during HRV infection period. Increased IRAK-M protein expression was confirmed in IRAK-M OE NCI-H292 cells (Fig. 1A). IRAK-M OE versus EV NCI-H292 cells consistently increased HRV-16 RNA levels at both 4 h (Fig. 1B) and 24 h (Fig. 1C) in a dose-dependent manner, particularly at 10^4 $TCID_{50}$/well where a greater percentage of cells was infected. To support HRV RNA data, we measured infectious viral particles released into the supernatants from cells infected with HRV-16 at 10^4 $TCID_{50}$/well by plaque assay. Like the RNA data, IRAK-M OE NCI-H292 cells had a higher viral titer starting at 4 h and reached a statistical significance at 24 h (Fig. 1D). Hence, 10^4 $TCID_{50}$/well was chosen as the HRV-16 infection dose for all other experiments in this study.

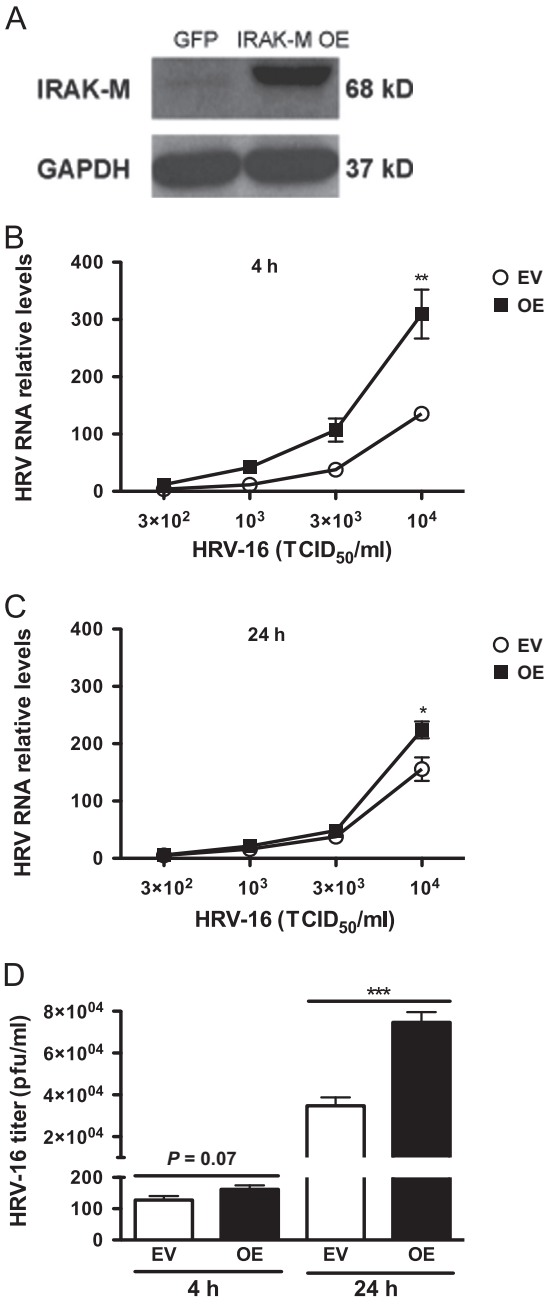

Fig. 1. IRAK-M over-expression promotes HRV-16 replication in human lung epithelial cell line. IRAK-M over-expressing (OE) and control (empty vector, EV) NCI-H292 cells were infected with various doses of HRV-16 or PBS for 4 and 24 h as described in Materials and methods. Cellular IRAK-M protein at the baseline of 4 h (A), HRV RNA levels at 4 h (B) and 24 h (C) post infection were determined by Western blot and quantitative RT-PCR, respectively. The released infectious viral particles (D) in supernatants of cells with HRV-16 infection at 10^4 $TCID_{50}$/well were determined by plaque assay. Data are expressed as means ± SEM ($n=3$). *$P<0.05$, **$P<0.01$, ***$P<0.001$.

To validate our findings in NCI-H292 cell lines, IRAK-M over-expression was performed in submerged human primary airway