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Porcine reproductive and respiratory syndrome virus activates inflammasomes of porcine alveolar macrophages via its small envelope protein E

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ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) infection results in extensive tissue inflammation and damage, which are believed to be responsible for increased susceptibility to secondary infection and even for death. However, its pathogenic mechanisms are not fully understood. To explore the mechanism underlying the PRRSV-induced tissue inflammation and damage, we investigated whether PRRSV activates porcine alveolar macrophage (PAM) inflammasomes which mediate por-IL-1β maturation/release and subsequently induce tissue inflammation and injury. Our results showed that PRRSV and its small envelope protein E significantly increased IL-1β release from LPS-primed PAMs; however, only PRRSV not protein E significantly increased IL-1β release from no-LPS-primed PAMs, which indicates PRRSV can activate inflammasomes of PAMs by its encoded protein E. These results provide a molecular basis for the pathogenic mechanism of PRRSV on inducing extensive tissue inflammation and damage, and suggest that the inflammasome may provide a potential therapeutic target for PRRS prevention and treatment.

Introduction

Porcine reproductive and respiratory syndrome (PRRS), caused by PRRS virus (PRRSV), is an acute infectious disease threatening swine production worldwide, characterized by the late-term reproductive failure in pregnant sows and respiratory disorder in piglets and young pigs (Lunney et al., 2010). So far, PRRS has not been effectively controlled.

Since its emergence in the late 1980s, many researches on PRRS etiology, epidemiology, pathogenic mechanisms, diagnosis and prevention have been extensively conducted (Nelsen et al., 1999; Meng et al., 1995; Hermann et al., 2007; Prickett et al., 2008b; Calzada-Nova et al., 2012)

Particularly, the pathogenic mechanisms based on virus–host interactions such as PRRSV antibody-dependent enhancement and immune suppression (Cancel-Tirado et al., 2004; Qiao et al., 2011) has been especially concerned. As we known, PRRSV mainly infects porcine macrophages, especially alveolar macrophages (PAMs) (An et al., 2010). Upon PRRSV infection, the PAMs are activated and induce the extensive inflammatory reactions and damage in PRRSV-infected tissues such as lungs and placenta (Thanawongnuwech et al., 2004), which are believed to be the most important pathogenic events leading to increase of susceptibility to secondary infection and even to death. Nevertheless, the mechanism by which PRRSV initiates PAM-activated inflammation is unknown. Recent studies, however, have revealed that macrophage-mediated tissue inflammation is related to a multiprotein complex called the inflammasome (Rathinam et al., 2012; McIntire et al., 2009; Kanneganti, 2010), which initiates innate immune responses by activation of caspase-1 protease, which processes pro-interleukin-1β (pro-IL-1β) and pro-interleukin-18 (pro-IL-18), removing the amino-terminal amino acids to release mature and active forms of the cytokines, the most significant inflammatory cytokines in triggering tissue inflammation (Dinarello et al., 2012).

The inflammasome, a multiprotein oligomer, consists mainly of caspase 1, apoptosis-associated speck-like protein containing CARD (ASC) and Nod-like receptors (NLRs) such as NLRP1, NLRP3, NLRC4, NLRP6 and NLRP12. NLRs constitute a family of

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intracellular pattern recognition receptors (PRRs) in monocytes/macrophages, which sense microbial molecules and endogenous "danger" signals released by injured cells.

Synthesis and processing of IL-1β are considered to require two distinct signals. One is the signal 1 involved in nuclear factor κB (NF-κB) activation leading to pro-IL-1β synthesis, triggered by various pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs) such as toll-like receptor (TLR) and retinoic acid-inducible gene 1 (RIG-I)-like receptors (RLRs). For example, lipopolysaccharide (LPS) can activate NF-κB through TLR4. Another is the signal 2 associated with inflammasome activation resulting in IL-1β and IL-18 processing and release, triggered by the conserved motifs of pathogens or cell danger signals following inflammasome/caspase-1 activation.

Whether or not PRRSV activates inflammasomes in macrophages and initiates extensive inflammatory reaction in virus-infected tissues has, until now, not been established. The work presented here provides evidence that this mechanism is operative through PRRSV-encoded protein E.

Results

PRRSV activates the inflammasomes of PAMs

To determine whether PRRSV activates the inflammasomes of PAMs, the PAMs were pre-treated with or without LPS and then infected with PRRSV (North American genotype PRRSV generated from the infectious clone pFL12) at different multiplicities of infection (MOI), the cell survival rates (Fig. 1A) at different time points postinfection were estimated by trypan blue exclusion, the IL-1β concentrations in the cell culture were measured by ELISA and caspase 1 in PAMs were detected by Western blot at 72 h post-infection. The Results showed that the cell survival rates decreased with the increase of the MOI and duration of PRRSV infection. The IL-1β concentration in the medium of LPS-primed or unprimed PAMs significantly increased proportionately with an increase in MOI ($P<0.05$~0.01) (Fig. 1B). Moreover, the IL-1β levels in the medium of LPS-primed PAMs was significantly higher than unprimed PAMs ($P<0.01$). This suggests that PRRSV promotes

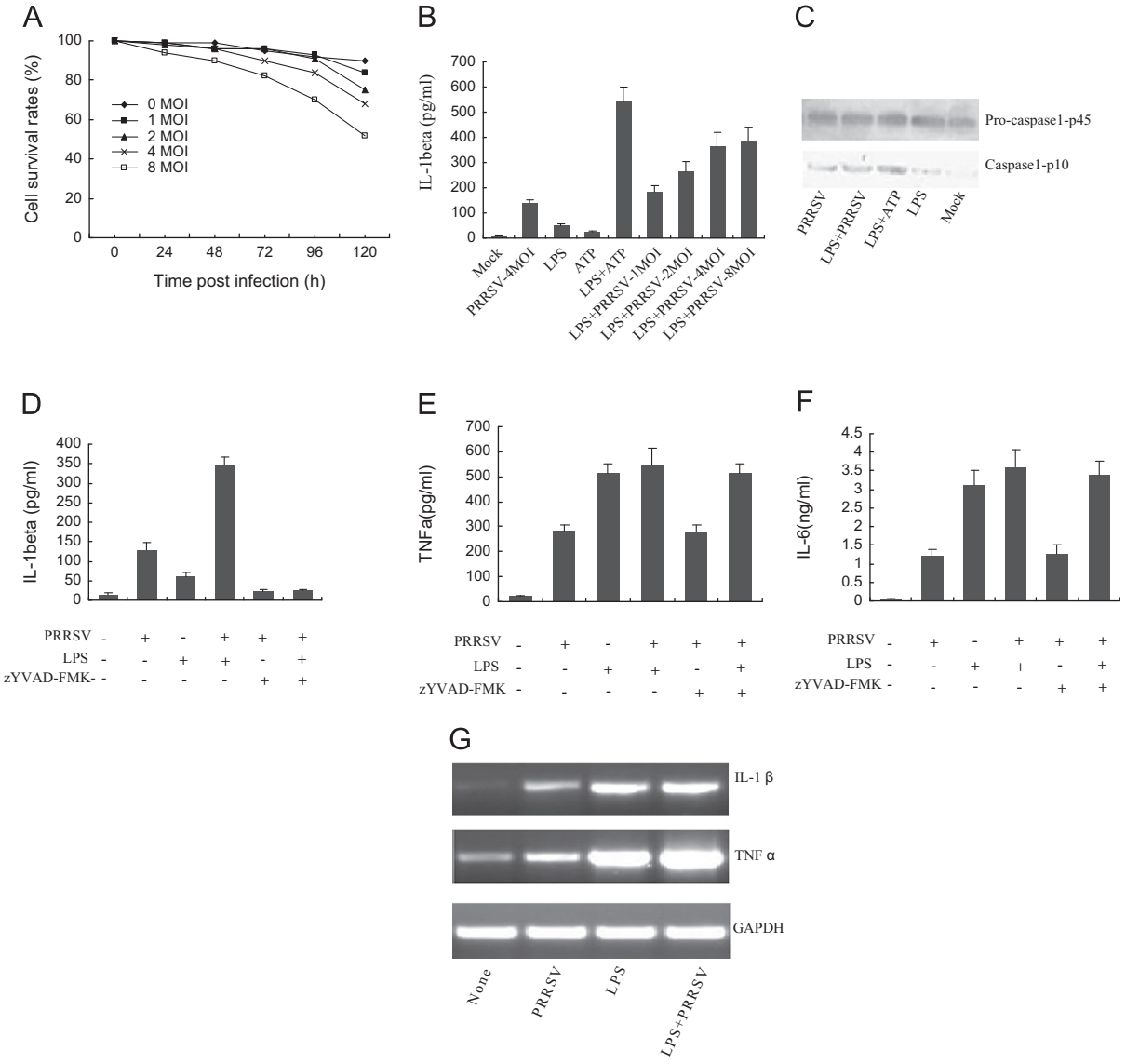

Fig. 1. PRRSV activation of inflammasomes of porcine alveolar macrophages. (A) Survival rates of PAMs after PRRSV infection at different MOI (1, 2, 4, 8); (B) Effect of PRRSV infection at the different MOI on IL-1β release from macrophages. (LPS+ATP group is a positive control of inflammasome activation). (C) immuno-blot detection for caspase 1 in PRRSV-infected PAMs. (D~F) Effect of zYVAD-FMK on IL-1β, TNFα and IL-6 release from PRRSV-infected macrophages, respectively. (G) IL-1β and TNFα mRNA levels in PRRSV-infected PAMs detected by RT-PCR.