Review Article

Innate Immune Responses against Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus

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Abstract | Antiviral innate immunity plays a central role at the early stage of viral life cycles. The highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) is a major concern for the swine industry and causes high degrees of morbidity and mortality. The intracellular signalling cascades of the innate immune system triggered by HP-PRRSV often lead to the elimination of infection. However, HP-PRRSV induces pathological changes and can cause aberrant immune responses in the host, resulting in acute lung injury and immune dysfunction. Studies of the innate immune responses to HP-PRRSV infection are crucial to gaining insight into HP-PRRSV-induced pathogenesis and for the development of novel protective vaccines. In this review, we discuss the innate immune responses against HP-PRRSV infection and suggest new areas of investigation into this important interface between pathogen and host.

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Introduction

Innate immune system is the first line of defence against viral infection and is also important for activating acquired immunity. Viral infection activates innate immunity through pattern recognition receptors (PRRs) that include Toll-like receptors (TLRs), (RIG)-I-like receptors (RLRs), NOD-like receptors (NLRs), and C-type lectin receptors (CLRs). Once the PRRs recognize conserved pathogen-associated molecular patterns (PAMPs), various intracellular signalling cascades are triggered to upregulate the expression of genes involved in innate immune responses (Takeuchi and Akira 2010). Type I interferons (IFNs) are an important group of antiviral cytokines that bind to the type I IFNs receptors on surrounding cells, to trigger signalling cascade that leads to the expression of interferon-stimulated genes (ISGs), to inhibit viral proliferation (Der et al., 1998; Maher et al., 2008).

The porcine reproductive and respiratory syndrome (PRRS) has caused huge economic losses to the swine industry worldwide (Allende et al., 2000). The causative agent of PRRS is porcine reproductive and respiratory syndrome virus (PRRSV), a small, enveloped, positive-sense, single-stranded RNA virus, belonging to the *Arteriviridae* family in the order *Nidovirales* (Conzelmann et al., 1993). In 2006, the highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV), with a unique discontinuous 30-amino acid deletion within its Nsp2 gene,

emerged in China and has caused substantial economic losses to the swine industry (Tong et al., 2007; Zhou et al., 2008). Thought to be a mutant belonging to the North American type (Sun et al., 2012), HP-PRRSV is still the most destructive swine pathogen currently in existence and additional HP-PRRSV isolates have been reported (Wang et al., 2013; Zhou et al., 2009). Compared to low-pathogenicity PRRSV isolates, HP-PRRSV exhibits much stronger tropism to porcine alveolar macrophages (PAM) and is highly virulent to pigs (Wang et al., 2014; Tong et al., 2007). Recent studies of HP-PRRSV have focused on the mechanisms by which the virus evades its recognition and elimination by the host innate immune responses.

Signalling pathways of innate immunity associated with HP-PRRSV infection

After PAMPs are sensed by PRRs, innate immune signalling pathways are triggered to antagonize virus infection. For RNA virus infection, two distinct PRRs, TLRs and RLRs, play central roles in the recognition of viral components. Accumulating evidence underscores the importance of TLRs in recognizing HP-PRRSV. To understand the roles of TLRs in eliciting host immunity, changes in the expression of the TLR2, 3, 7, and 8 genes in PAMs from HP-PRRSV infected piglets were evaluated using real-time PCR. TLR3, 7, and 8 transcripts were significantly up-regulated in PAMs infected with HP-PRRSV, as compared with PAMs infected with low pathogenic PRRSV (Zhang et al., 2013a). However, these studies were limited to analyzing transcript abundance and changes to protein expression were not evaluated. Activation of TLR3 with dsRNA *in vitro* suppresses PRRSV infectivity in PAMs and targeting TLR3 with small-interfering RNA resulted in an increase in PRRSV infectivity, indicating the importance of TLR3 to the host response to PRRSV infection. By contrast, treating PAMs with lipopolysaccharide (LPS) to activate TLR4 signalling did not affect PRRSV infectivity (Miller et al., 2009; Sang et al., 2008). TLR3 and TLR7/8 ligands have been used as immunostimulatory adjuvants and enhance the protective effects of vaccination against PRRSV (Zhang et al., 2013b). Thus, TLR3 and TLR7/8 play important roles in anti-PRRSV innate immunity by providing early innate protection.

While no data are available about the roles of RLRs in antagonizing HP-PRRSV infection, the transcription factors NF-κB and AP-1, which are mapped downstream of RIG-I/MDA5, are activated by HP-PRRSV infection (Chen et al., 2014). However, activation of these factors also regulates the production of inflammatory cytokines, which may contribute to the development of pathological lesions.

PRR activation leads to enhanced expression of several transcription factors that subsequently trigger type I IFN synthesis, an important group of antiviral cytokines (Luo et al., 2011). Interferon signalling is crucial for transcriptional regulation during HP-PRRSV infection (Badaoui et al., 2014) and many studies have shown that type I IFNs inhibit PRRSV proliferation (Luo et al., 2011; Albina et al., 1998a; Overend et al., 2007). Polyinosinic–polycytidylic acid (poly (I:C)) also inhibits HP-PRRSV replication in MARC-145 cells by upregulating the expression of the interferon (IFN)-induced protein with tetratricopeptide repeats 3 (IFIT3) (Zhang et al., 2013c), and antiviral protein IFIT3 also up-regulated by IFN-α (Liu et al., 2011; Schmeisser et al., 2010).

While the mechanism of the antiviral effect against HP-PRRSV by type I IFNs has not been fully elucidated, the IFN-induced genes are thought to play important roles. Recently, the gene expression profile of HP-PRRSV-infected PAMs was analysed. Another type I IFNs inducible protein, Mx1, was confirmed to be upregulated by HP-PRRSV infection, suggesting that Mx1 may mediate a specific antiviral state against HP-PRRSV infection (Xiao et al., 2015). Previous studies revealed that the interferon-inducible transmembrane proteins (IFITMs) exert antiviral activity against various viruses (Brass et al., 2009), but overexpression of IFITM1 did not have an obvious effect on HP-PRRSV replication in MARC-145 cells (Wang et al., 2014). Further studies are needed to identify the ISGs and other antiviral genes relevant to controlling HP-PRRSV.

Innate immune restriction factors in HP-PRRSV infection

Relatively little is known about the effects of intrinsic restriction factors on HP-PRRSV infection. Host restriction factors are an important part of innate immunity and act as the first line of defense against viral infections. These factors, such as TRIM5α, APOBEC3G, Tetherin, and SAMHD1, are constitutively expressed in host cells to fight against invading pathogens (Stremlau et al., 2004; Sheehy et al., 2002;

Neil et al., 2008; Goldstone et al., 2011). To date, only the effects of Tetherin and SAMHD1 on HP-PRRSV infection have been confirmed. SAMHD1 overexpression reduced HP-PRRSV replication in MARC-145 cells by inhibiting the synthesis of the viral complement RNA. SAMHD1 protein expression is significantly upregulated in PAMs infected by HP-PRRSV (Yang et al., 2014). It is uncertain whether SAMHD1 activity is blocked and why SAMHD1 expression is induced by HP-PRRSV infection, and these questions warrant further investigation. Conversely, overexpression of Tetherin had no obvious effects on HP-PRRSV replication in MARC-145 cells (Xiao et al., 2010). Whether HP-PRRSV counteracts the antiviral activities of Tetherin is unclear.

Immune evasion strategies of PRRSV

At present, studies of PRRSV are focused on the mechanisms by which the virus modulates innate immunity to evade its recognition and elimination by host innate immune responses. Both *in vivo* and *in vitro*, type I IFNs are suppressed in HP-PRRSV-infected cells, suggesting that some aspect of the host innate immune response is blocked (Xiao et al., 2010; Albina et al., 1998b; Buddaert et al., 1998). Several PRRSV proteins have been identified as IFN antagonists, the Nsp1, Nsp2, Nsp4, Nsp11, and N proteins (Xiao et al., 2015). PRRSV infection is thought to inhibit type I IFN production by suppressing the phosphorylation and nuclear translocation of IRF-3, an effector of the RIG-I signaling pathway (Beura et al., 2010; Luo et al., 2008; Sagong et al., 2011). However, a different study suggested that PRRSV infection did not block IRF-3 phosphorylation and nuclear translocation, but rather inhibited the association of IRF-3 with the CREB-binding protein (CBP) in the nucleus, leading to reduced IFN responses (Kim et al., 2010). PRRSV infection can also inhibit the induction of type I interferon genes by blocking the transduction of the JAK/STAT signal pathway (Wang et al., 2013; Patel et al., 2010). It is of interest to explore in greater detail the strategies utilized by HP-PRRSV to evade innate immune responses.

Prospects

Some degree of success has been achieved in managing host immune responses to reduce the burden of HP-PRRSV infections. However, many key innate immune signalling pathways and interactions between HP-PRRSV and the host have yet to be explored. A greater understanding of the interactions between HP-PRRSV and host innate immunity is expected to provide new insights into HP-PRRSV virulence mechanisms and may suggest efficacious strategies for vaccine and antiviral development to reduce the enormous burden of this important pathogen on the swine industry.

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