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Short Communication

An attenuated EIAV strain and its molecular clone strain differentially induce the expression of Toll-like receptors and type-I interferons in equine monocyte-derived macrophages

Jian Ma, Shan-Shan Wang, Yue-Zhi Lin, Hai-Fang Liu, Hua-Mian Wei, Cheng Du, Xue-Feng Wang, Jian-Hua Zhou *

State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, 427, Maduan Street, Nangang District, Harbin 150001, China

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ABSTRACT

Activations of endosomal TLRs include TLR3, TLR7/8, and TLR9 stimulates the production of cytokines, such as type I interferons (IFNs), and therefore involves in virus–host interactions. In the present study, two equine anemia virus (EIAV) strains $EIAV_{FDDV13}$ and $EIAV_{FDDV}3–8$, which showed different induction on protective immunity, were compared regarding their ability to regulate the expression of endosomal TLRs, as well as type I IFNs, after infection of equine monocyte-derived macrophages (eMDMs). Our results showed that $EIAV_{FDDV13}$ dramatically up-regulated the expression of TLR3 and IFNβ and less robustly up-regulated the expression of TRL9 and IFNα1, whereas $EIAV_{FDDV}3–8$ induced significantly lower expression of type I IFN mRNA and protein and more strongly down-regulated the expression of TLR7 and TLR8. In addition, no significant differences in cell apoptosis were observed between these two strains. Given that the genomic variation of $EIAV_{FDDV13}$ is considerably higher than that of molecular clone $EIAV_{FDDV}3–8$, our results suggest that stronger TLR3 activation and increased INFβ production induced by the multi-species strain are associated with an effective vaccine-elicited protective immune response.

1. Introduction

In the early stage of viral infection, activation of the innate immune system represents the first line of defense, in terms of viral resistance and clearance. Pattern recognition receptors (PRRs) expressed by host immune cells can recognize evolutionarily conserved pathogen-associated molecular patterns (PAMPs) on invasive pathogens and initiate effective and appropriate anti-viral responses, thereby making PRRs a crucial component of innate immune activation (Akira et al., 2001; Janeway and Medzhitov, 2002; Medzhitov, 2007). Following the initial recognition of pathogens, host cells react by producing chemokines, inflammatory factors, adhesion molecules, type I interferons (IFNs, includes IFNα1 and IFNβ) and other cytokines. These molecules promote an inflammatory environment and help to establish the subsequent adaptive immune response (Akira et al., 2001; Iwasaki and Medzhitov, 2010; Kawai and Akira, 2006). Toll-like receptors (TLRs) and RIG-I-like receptors (RLRs) are the two main types of PRRs, with significant roles in activating innate immunity. TLR3, TLR7/8 and TLR9, in the TLR family, and RIG-1 and MDA5, in the RLR family of immune-receptors, are crucial participants in the activation of innate immune responses to viral pathogens. TLR3, RIG-I and MDA5 are specifically activated by double-stranded RNA (dsRNA), TLR7 and TLR8 are specifically activated by single-stranded RNA (ssRNA), and TLR9 is activated by DNA (Kawai and Akira, 2006; Mogensen et al., 2010). Once

* Corresponding author. Tel.: +86 189 46066124; fax: +86 451 51997166.
E-mail address: jianhua_uc@126.com (J.-H. Zhou).

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a PRR is activated by the appropriate PAMPs, downstream signaling pathways are activated, and the expression of inflammatory factors and type I IFNs is then initiated via the activation of transcription factors, such as nuclear factor-kB (NF-kB) and IFN-regulatory factors (IRFs) (Kawai and Akira, 2008; Tailor et al., 2006). Type I IFNs are the major downstream products of TLR activation. TLR3 activation primarily produces IFNβ, whereas TLR7/8 and TLR9 activation generates IFNα (Baccala et al., 2007). In addition to direct antiviral activity, type I IFNs participate in the regulation of specific immune responses at several levels, including host antiviral immune recognition, cellular immunity and humoral immunity. PRR activation and type I IFN production coordinate the activation of the innate immune system and the establishment of adaptive immune responses.

Equine infectious anemia virus (EIAV) is a lentivirus that infects animals within the Equidae family and demonstrates macrophage tropism (Leroux et al., 2004). EIAV infections cause equine infectious anemia (EIA), which clinically presents in acute, chronic and asymptomatic phases. The acute and chronic phases are associated with viremia accompanied by high fever, anemia, thrombocytopenia, edema and weight loss. Most infected horses become life-long asymptomatic carriers of the virus after 8–12 months and exhibit a low level of viral replication in tissues rich in mononuclear cells. In contrast to host infection with other lentiviral species, greater than 90% of EIAV-infected horses enter an asymptomatic carrier state, due to the immune system's ability to control viral replication within the infected host. The ability of the immune system to control EIAV replication presents a unique lentiviral model for studying the key immune factors that inhibit lentiviral replication. In addition, the majority of asymptomatic EIAV carriers are effectively immunized to subsequent infection by other EIAV strains (Craigo et al., 2007), suggesting that the EIAV system can be used as a model for studying the immune responses that are essential for protection and clearance of lentiviral infection.

In a recent study, an attenuated EIAV strain, $EIAV_{FDDV13}$, and a proviral derivative of this attenuated strain, $EIAV_{FDDV}3–8$, were compared regarding their ability to induce protective immunity(Ma et al., 2011). Although these two strains replicated equally well in vitro and in vivo, the proviral strain induced significantly less protection from disease and infection caused by viral challenge and significantly lower specific neutralizing capability. It is known that the activation of innate immunity is correlated with the development of adaptive immune responses (Iwasaki and Medzhitov, 2004, 2010; Kawai and Akira, 2006); therefore, differences in the activation of a panel of innate immune responses between these two EIAV strains were examined.

2. Methods and materials

2.1. Cells and viral strains

Macrophages are the primary host cell target population for EIAV and are the major subset of immune cells responsible for recognizing, processing and presenting EIAV viral antigens to elicit an effective immune response (Hume, 2008; Leroux et al., 2004). So, equine monocyte-derived macrophages (eMDMs) were used as target cells for infection by these two EIAV strains in this study. eMDMs were prepared from horse peripheral blood mononuclear cells (PBMC). Supernatant plasma was obtained from freshly collected, heparinized whole horse blood following precipitation at room temperature for 30 min. The blood cells in the plasma were isolated after 1,000 rpm centrifugation. Following 2–3 washes with PBS (Sigma, USA), the cells were incubated in RPMI 1640 culture medium (Gibco, USA) supplemented with 10% horse serum (Hyclone, USA) and 40% fetal bovine serum (FBS) (Hyclone, USA) at 37 °C with 5% CO_2. After 24 h of incubation, non-adherent and loosely adherent cells were removed with PBS washing. The remaining adherent cells, the majority of which were eMDMs, were detached with normal saline and seeded into microplates (Costar, USA).

Two EIAV strains $EIAV_{FDDV13}$ and $EIAV_{FDDV}3–8$ were used in this study. $EIAV_{FDDV13}$, an attenuated vaccine strain of EIAV(Jiang et al., 2011; Ma et al., 2009). $EIAV_{FDDV}3–8$, an infectious clone strain derived from the proviral DNA of $EIAV_{FDDV13}$(Ma et al., 2011).

2.2. Quantification of EIAV load and detection of viral replication in eMDMs

The infectious titer of these two EIAV strains was tested by measuring the median tissue culture infective dose method (TCID50) as described before (Raabe et al., 1998). Real time quantitative PCR (qPCR) was used to identify the viral nucleotides of EIAV in eMDMs according to previously described procedures (Ma et al., 2011).

To examine the proliferation profiles of EIAV in eMDMs, 1×10^5 cells were infected with 1×10^3 TCID50 of $EIAV_{FDDV13}$ or $EIAV_{FDDV}3–8$ in a 96-well microplate. The cells, which were used to detect the intracellular viral RNA or DNA copies after infection by EIAV for 3, 6, 12, 24, 36, 48, and 72 h, were washed by PBS at different detection times post infection and treated with trypsin to the virus adhered but not entered into the cells. Triplicate wells were used for each detection time point. Total RNA and DNA was exacted from the harvested cells using Trizol (Invitrogen, USA) for qPCR analysis of the viral genomic RNA and DNA. The replication dynamics of the viruses were determined in three independent experiments.

2.3. Measurement of mRNA expression by the branched DNA technique

The eMDMs in 24-well plates were infected with these two strains at 5×10^3 TCID50 per infection. The mRNA expression levels of TLR3, TLR7/8 and TLR9 were determined at 3 h, 6 h, 12 h, 24 h and 36 hpi. Because type I IFNs are the main downstream products of TLR activation (Kawai and Akira, 2006; Severa and Fitzgerald, 2007), the expression of type I IFNs was monitored at 48 h and 72 hpi in addition to the sampling time points evaluated for TLRs. Cells were collected at the indicated time points and stored