Veterinary Immunology and Immunopathology 195 (2018) 76–83

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

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

Short communication

Saponin Quil A up-regulates type I interferon-regulated gene and type I and II interferon expressions which are suppressed by porcine reproductive and respiratory syndrome virus

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ARTICLE INFO

Keywords:
Porcine reproductive and respiratory syndrome virus
Quil A
Innate immunity
Interferon
Inflammatory cytokines

ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) suppresses innate immune response following infection of myeloid antigen-presenting cells. Poor innate immune response results in weak and delayed PRRSV-specific adaptive immunity, and facilitates PRRSV replication, pathogenesis, and persistent infection. Numerous efforts have been made to enhance the effective innate and adaptive immune defenses to PRRSV, however, only a few attempts have so far elicited satisfactory results. The present study aims to evaluate in vitro the potential of saponin quil A to enhance the expression of type I interferon (IFN)-regulated gene, type I and II IFNs, and pro-inflammatory cytokines in PRRSV-inoculated peripheral blood mononuclear cells (PBMC). Naïve PBMC from four PRRSV-seronegative pigs were inoculated with PRRSV and subsequently stimulated with quil A in the absence or presence of either polyinosinic:polycytidylic acid (poly IC) or lipopolysaccharide (LPS). The mRNA expression levels of myxovirus resistance 1 (Mx1), interferon regulatory factor 3 (IRF3), IRF7, 2′-5′-oligoadenylatesynthetase 1 (OAS1), stimulator of interferon genes (STING), osteopontin (OPN), IFNα, IFNβ, IFNγ, interleukin-2 (IL-2), IL-10, IL-13, tumor necrosis factor alpha (TNFα), and transforming growth factor beta (TGFβ) were evaluated by real-time PCR. Compared with uninoculated PBMC, PRRSV significantly suppressed expression of all immune parameters except IL-2, IL-10, IL-13, and TGFβ. When compared with PRRSV-inoculated PBMC, stimulation with quil A significantly enhanced Mx1, IRF3, IRF7, OAS1, STING, IFNβ, and IFNγ mRNA expressions, and significantly reduced TGFβ mRNA expression. Our findings thus suggest that quil A has a potential to up-regulate the expression of type I IFN-regulated gene and type I and II IFNs which are suppressed by PRRSV. Therefore, it may serve as an effective immunostimulator for potentiating the innate immune defense to PRRSV.

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) causes serious economic loss in swine industry worldwide. The virus is an enveloped positive-sense single-stranded linear RNA virus, belonging to the family Arteriviridae. PRRSV genome is approximately 15 kb in size, and comprises 10 open-reading frames (ORFs), designated ORF1a, 1b, 2a, 2b, 3, 4, 5a, 5, 6, and 7. PRRSV ORF1a and ORF1b encode 14 nonstructural proteins (nsp), while ORF2-7 encode eight structural proteins, i.e. glycoprotein 2a (GP2a), 2b protein, GP3, GP4, ORF5a protein, GP5, matrix (M), and nucleocapsid (N), respectively (Kappes and Faaberg, 2015). Both nsp and structural proteins play important roles in PRRSV evasion of host immune defense (Lunney et al., 2016).

PRRSV strains are divided into European (type 1) and North American (type 2) genotypes. Within the type 1 PRRSV genotype, three subtypes have been delineated. Type 1 PRRSV subtype 3 strains are more pathogenic than the other two subtypes. Type 2 PRRSV strains consist of recently emerged highly pathogenic strains called “HP-PRRSV” which caused serious outbreaks in China and Southeast Asian countries. PRRSV of all strains infects myelomonocytic cell lineage, including monocytes, macrophages, and dendritic cells. Following infection, the virus induces poor innate and adaptive immune responses. In PRRSV-infected cells, the expression of type I and II interferons (IFNs) was barely detected (Albina et al., 1998; Meier et al., 2003), and the expression of pro-inflammatory cytokines, e.g. tumor necrosis factor alpha (TNFα) and interleukin 1 (IL-1) was relatively low, compared to those seen after infection with other pathogens (Lopez-Fuertes et al., 2000; Van Reeth et al., 1999). Other innate immune activities including phagocytosis, production of reactive oxygen species, antigen

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https://doi.org/10.1016/j.vetimm.2017.11.009
Received 25 July 2017; Received in revised form 27 October 2017; Accepted 28 November 2017

presentation, T cell activation, and microbial killing activity of PRRSV-infected cells were reduced (Charerntantanakul and Kasinrerk, 2012; Sang et al., 2011). In in vivo studies, PRRSV-specific cytotoxic T lymphocytes (CTL) and T helper 1 (Th1) cells as well as neutralizing antibodies (NAb) appeared in peripheral blood and serum approximately three to four weeks after infection (Meier et al., 2003). These were considered delay compared to similar responses to other swine viral pathogens, e.g. swine influenza virus and transmissible gastroenteritis virus (Thacker, 2001). The population of CTL and Th1 cells and the titer of NAb were slowly increased over a period of months (Meier et al., 2003). Delayed and weak adaptive immune response to PRRSV was suggested to be attributed, at least in part, to a weak innate immune response to the virus (Loving et al., 2015).

In contrast to immune responses to most PRRSV strains, immune responses to type 1 PRRSV subtype 3 and HP-PRRSV are faster and stronger in terms of antibody and cell-mediated immunity (CMI) as well as inflammatory cytokine expressions e.g. IL-1, IL-12, TNFα, IFNα, and IFNγ. This is mainly due to the increased infectivity and replication efficiency of these virus strains in vivo. Other mechanisms involving in an improved immune response to these PRRSV strains have not been clearly identified. The stronger immune responses may have supported an enhanced or faster clearance of virus in tissues, compared to most PRRSV strains (Li et al., 2016; Weesendorp et al., 2014).

Mechanisms involving in poor innate immune response to PRRSV have been studied. These reportedly include PRRSV suppression of signaling molecule and transcription factor activation, e.g. retinoic acid induced gene-1 (RIG-1) (Luo et al., 2008), mitochondrial antiviral signaling protein (MAVS) (Sun et al., 2016), interferon regulatory factor 3 (IRF3) (Beura et al., 2010), nuclear factor kappa B (NFκB) (Wang et al., 2015), and signal transducer and activator of transcription 1 (STAT3) (Yang et al., 2017), and extracellular signal-regulated kinase (ERK) (Hou et al., 2012); PRRSV suppression of pattern recognition receptor (PRR) expression, e.g. Toll-like receptor 3 (TLR3) and TLR7 (Chaung et al., 2010); PRRSV-mediated degradation of signaling molecules, i.e. CREB-binding protein (CBP) (Kim et al., 2010); PRRSV inhibition of nuclear translocation of transcription factors, e.g. STAT1 and STAT2 (Yang and Zhang, 2016), and PRRSV up-regulation of anti-inflammatory cytokine, i.e. IL-10 (Charerntantanakul and Kasinrerk, 2010, 2012). These mechanisms have been reported to mediate, at least in part, through PRRSV nsp1 (Beura et al., 2010), nsp2 (Sun et al., 2010), nsp4 (Huang et al., 2016), nsp5 (Yang et al., 2017), nsp11 (Sun et al., 2016), GP5 (Zhixuan et al., 2015), and N proteins (Sagong and Lee, 2011).

Efforts to enhance immune response to PRRSV have been made. These include the use of various forms of PRRSV vaccines, e.g. modified-live virus, inactivated virus, gene-deleted, vectored, DNA, and subunit vaccines (Charerntantanakul, 2012), in combination with various types of adjuvants, for example, pro-inflammatory cytokines, e.g. IL-1, IL-12, and granulocyte-macrophage colony-stimulating factor (GM-CSF); chemical reagents, e.g. polyinosinic:polycytidylic acid (poly IC), poly IC with polylysine and carboxymethylcellulose (poly ICLC), and poly(lactic-co-glycolic) acid (PLGA); bacterial components, e.g. CpG oligodeoxynucleotides (ODN), cholera toxin, and lipopolysaccharide (LPS); immunostimulatory proteins, e.g. C3d, CD40 ligand, and peptide nanofiber hydrogel; and commercial adjuvants, e.g. Montanide™ Gel 01 ST (Binjawadagi et al., 2014; Charerntantanakul, 2009; Li et al., 2013; Tabynov et al., 2016). Some of those efforts efficiently improve immune response and vaccine efficacy against homologous PRRSV challenge, but only a few of them, e.g. PLGA, CpG ODN, peptide nanofiber hydrogel, and Montanide™ Gel 01 ST reportedly improve immune response and vaccine efficacy against heterologous PRRSV challenge (Binjawadagi et al., 2014; Charerntantanakul, 2009; Li et al., 2013; Tabynov et al., 2016). This small number of achievement suggests the need of further investigations on other potential immunostimulators for PRRSV, particularly for cross-protection against heterologous challenge with varying field PRRSV isolates.

Quil A is a triterpenoid saponin from the bark of South American tree, *Quillaja saponaria* Molina. It has been reported to possess immunostimulatory effects, particularly on stimulation of CTL, Th1 cells, and antibody responses in human and veterinary experimental vaccines (Sun et al., 2009a). Its mechanism of immunostimulation is not well identified. In pigs, quil A has been reported to enhance serum and mucosal IgA production and protective efficacy of *Actinobacillus pleuropneumoniae* inactivated and subunit vaccines (Willson et al., 1995); NAb production and protective efficacy of inactivated swine influenza H3N2 virus vaccine (Bikour et al., 1996); antibody production and protective efficacy of *Taenia solium* TSOL18 vaccine (Assana et al., 2010), *Streptococcus suis* Sao vaccine (Li et al., 2007), and *Toxoplasma gondii* crude rhoptry vaccine (da Cunha et al., 2012); protective efficacy of *T. solium* TSOL16-TSOL18 fusion vaccine (Jayashi et al., 2012); and antibody production of foot-and-mouth disease virus (FMDV) vaccine (Xiao et al., 2007) and *S. suis* recombinant SsnA vaccine (Gomez-Gascon et al., 2016).

When combined with cholesterol and phospholipids to form immunostimulatory complexes (ISCOMs), quil A can activate both CMI and antibody responses to a broad range of viral, bacterial, and protozoal antigens (Sun et al., 2009b). In pigs, quil A-integrated ISCOMs have been reported to induce IFNβ, TNFα, and osteopontin (OPN) but not IFNα expression in porcine peripheral blood mononuclear cells (PBMC) (Fossum et al., 2014) and OPN expression in injected muscle (Ahlberg et al., 2012). In addition, quil A-integrated ISCOMs reportedly enhance lymphocyte proliferation, antibody production and protective efficacy of pseudorabies virus (PRV) subunit vaccine (Tulman and Garmendia, 1994) and live *Mycoplasma hyopneumoniae* vaccine (Maes, 2014); protective efficacy and antibody production of *T. gondii* crude rhoptry vaccine (Garcia et al., 2005) and FMDV recombinant C-terminal VP1 vaccine (Bayry et al., 1999); and antibody production of enterotoxigenic *Escherichia coli* fimbriae vaccine (Nagy et al., 1990).

The present study evaluates the immunostimulatory effects of quil A on the expression of type I IFN-regulated genes, type I and II IFNs, and inflammatory cytokines in porcine PBMC in response to PRRSV. Our findings provide useful evidence for further exploitation of quil A as immunostimulator for PRRSV and possibly for other swine vaccines.

2. Materials and methods

2.1. PRRSV

MARC-145 cells and HP-PRRSV (isolate CUVDL 3/1/4; isolated from lung lavage) were a courtesy of the veterinary diagnostic laboratory, faculty of veterinary science, Chulalongkorn university, Thailand. Both cells and viruses were propagated in MEM^{++} comprising MEM (Caisson Laboratories, Smithfield, UT), 10% heat-inactivated FBS (Capricorn Scientific GmbH, Germany), penicillin (100 IU/ml), streptomycin (100 μg/ml), and amphotericin B (250 ng/ml) (all from Gibco, Grand Island, NY). The virus cultures were frozen and thawed twice, centrifuged, and the supernatants were collected, filtered through 0.22 μM filter (Minisart®, Sartorius, France), and kept at −80 °C. The virus titer was determined by immunoperoxidase monolayer assay, using primary mouse mAbs specific for PRRSV N proteins of both type 1 and type 2 genotypes (IgG2b, clone 5C61) (Median Diagnostics, South Korea) and secondary horseradish peroxidase-conjugated goat anti-mouse IgG antibodies (CiteAB, UK). The virus titer was adjusted to 10^6 $TCID_{50}$/ml. The passage number of PRRSV used in this study was at passage ninth in MARC-145 cells.

2.2. Quil A

Quil A (cat# vac-quil, Invivogen, San Diego, CA) was resuspended with sterile water to 10 mg/ml. The solution was filtered through 0.22 μM filter (Minisart®), aliquoted, kept at −20 °C, and protected from light until use. The presence of LPS in the resuspended quil A