PRRSV vaccination in pig enhances the subsequent lipopolysaccharide-induced cytokine expression in the peripheral blood mononuclear cells

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Summary

To elucidate the crucial interaction between porcine reproductive and respiratory syndrome virus (PRRSV) and bacterial lipopolysaccharides (LPS) in the development of host immunity, the present study investigated the effect of in vivo PRRSV vaccination on LPS-induced cytokine mRNA expression patterns in the peripheral blood mononuclear cells (PBMCs). The PBMCs were isolated from the whole blood samples collected at before vaccination (D0), and day 1 (D1), 14 (D14) and day 28 (D28) post primary PRRSV vaccination in Pietrain pigs. The PBMCs collected at each time point were then stimulated with LPS (@ 0.5 ug/ml) for 24 h in vitro. The relative mRNA expressions of IFNγ, TNFα, IL1β, IL10, TLR4, NFkB, IRF3 and MyD88 were quantified by qRT-PCR followed by protein measurement of IL-1β and TNF-α in the cell culture supernatants using by ELISA. PRRSV vaccination resulted in upregulation of IL1β and MyD88 while LPS stimulation increased the TLR4 expression in the naïve PBMCs. Although, PRRSV vaccination induced an upward trend of TNFα, IFNγ, IL1β, TRL4 and NFkB expression, subsequent in vitro LPS stimulation to the vaccine-primed PBMCs resulted in more pronounced expression of these cytokines. We concluded that the PRRSV vaccination in pigs is permissive to PBMCs and may sensitize them in such way that enhances the cytokine mRNA expression upon subsequent exposure to LPS.

Keywords: PRRSV-LPS interaction, innate immunity, PBMCs, cytokines, pig

Introduction

The porcine reproductive and respiratory syndrome virus (PRRSV) is one of the leading viral pathogens involved in the porcine respiratory disease complex. PRRSV-infected pigs mostly suffer from respiratory distress and reproductive failure, and are highly susceptible to secondary bacterial infections (van Gucht et al. 2003). The pathogenesis of PRRSV begins with the suppressing of the host's anti-viral defenses to control replication of the virus, which resulted from modulation of the early warning components of the immune system (Koyama et

al. 2008). Innate immune response started once the conserved microbial structures, pathogen-associated molecular patterns (PAMPs) such as bacterial cell-wall components and viral nucleic acids, are recognized by the pattern-recognition receptors (PRRs) of host immune cells (Koyam et al., 2008). LPS has been used as a PAMP to mimic the innate immune response to in vitro bacterial infection in PBMCs (Uddin et al. 2012). LPS-induced exacerbation of PRRS disease severity has been documented by Labarque et al. (2002) and the PRRSV–LPS combination has been considered a reproducible experimental model to study multifactorial respiratory disease in porcine alveolar macrophages (Van Gucht et al. 2004). Because of sampling conveniences and reflectic nature of whole-body immunity, we have studied the porcine innate immune responses after in vitro LPS stimulation (Uddin et al. 2012)http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4093518/ as well as after in vivo PRRSV vaccination (Islam et al. 2016; Islam et al. 2017) using the PBMCs model. Herein, we hypothesized whether priming of PBMCs by in vivo PRRSV vaccination in pigs influences the recognition and signal transduction induced by secondary bacterial infection. However, little is known about immunogenic mechanisms of interaction between exposure of primary PRRSV vaccine and the subsequent bacterial invaders. Taken together, the present study aimed to investigate the LPS-induced cytokine expression patterns in the PBMCs obtained from pigs immunized with PRRSV vaccine.

Material and methods

Three female Pietrain piglets were immunized with live attenuated PRRSV vaccine with a primary dose at 28 days and a booster dose at 56 days of age. Whole blood samples were collected through venipuncture before vaccination (D0), day 1 (D1), 14 (D14) and day 28 (D28) post primary vaccination. The PBMCs were separated from whole blood using Ficoll-Paque (Histopaque®-1077) density gradient centrifugation as described by Islam et al. (2017). Purified PBMCs with a concentration of 1×10^6 cells/ml were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, 1% antibiotic and 1% antimycotics. Cells were stimulated with LPS (@ 0.5 ug/ml) and incubated for 24 h at 37˚C with 5% CO_2.

Total RNA was extracted from cultured PBMCs by using the miRNeasy mini kit (Qiagen, Co.,) along with on column DNase treatment according to the manufacturer's protocol. The cDNA probes were prepared by using First Strand cDNA Synthesis Kit (Thermo Scientific™, Co.,). The iTaq™ Universal SYBR® Green Supermix-based reactions were amplified by the StepOnePlus™ Real-Time PCR System (Applied Biosystems®, Germany). Gene-specific expression was measured as relative to the geometric mean of the expression of two housekeeping genes (GAPDH and ACTB). The delta Ct (ΔCt) [$\Delta Ct = Ct_{target} - Ct_{housekeeping\ genes}$] values were calculated as the difference between target gene and reference genes and expression was calculated as $2^{(-\Delta\Delta Ct)}$. The mean differences of relative expression between treated and control groups were analyzed by standard one-way ANOVA as well as repeated measures ANOVA test using the GraphPad prism v.5.3. The significance level was set as to $p < 0.05$.

Results and discussion

In vivo PRRSV vaccination caused a significant ($p < 0.05$) increase in the mRNA expression of IL1β, MyD88 and NFkB but a significant decrease in IFNγ mRNA expression level of PBMCs at 24 h post vaccination (Fig 1). The in vitro LPS stimulation of PBMCs showed a significantly ($p < 0.05$) increased level of TLR4 expression after 24 h of incubation. The in vivo PRRSV vaccination causes significantly MyD88 expression in PBMCs compared to that of in vitro LPS stimulation. On the other hand, TLR4 expression was significantly higher in LPS stimulated PBMCs than that of PRRSV vaccinated ones. There was a decreasing trend of IRF3, TNFα and IL10 expression level in vaccinated PBMCs but an increasing trend was observed for TNFα and IL10 expression levels in LPS stimulated PBMCs (Fig 1).

The PRRSV vaccine-induced IFN response was much higher in PBMCs collected at D28 compared to that of D1 and D14 of vaccination (Fig 2A). The TLR4, NFkB and TNFα expression also showed an increasing trend over the time of post PRRSV vaccination (Fig 2A,B). The expression of IL1β and MyD88 showed upward trend at D1 post vaccination followed by decreasing trend over the D14 and D28 of vaccination. The IL10 expression in PBMCs also showed a decreasing time from D1 over D28 of PRRSV vaccination.

Lipopolysaccharide treatment of PBMCs isolated from PRRSV vaccinated pigs showed an overall scenario that synergistic effect of LPS and PRRSV antigen induced more prominent cytokine responses than that of LPS or PRRSV alone (Fig 3). TNFα expression was increased after LPS stimulation at D1 and D14 post vaccination, TLR4 expression was enhanced with LPS stimulation to the PBMCs of D14 and D28 vaccinated pigs. LPS induced elevation of IFNγ expression in PBMCs was noted at D28 days post vaccination whereas a significant increase of IL1β and MyD88 were observed at D1 post vaccinated PBMCs. The expression of NFkB was upward at all three post vaccination time points. On the contrary, IRF3 expression was decreased in PRRSV vaccine-primed PBMCs upon subsequent exposure of LPS in vitro (Fig 3).

The PAMP-PRR interaction initiates the release of cellular cytokines and chemokines which in turn switch on transcription factors including interferon regulatory factors (IRFs) and nuclear factor kappaB (NFkB), and their adaptor proteins (e.g., MyD88) subsequently resulted proinflammatory responses (Koyama et al. 2008). Though, PRRSV and LPS are recognized initially by different PRRs, they shared the downstream innate immune signaling cascades, thus vaccine-pulsed PBMCs respond rapidly and in a stronger manner as compared to naïve PBMCs. The current study revealed that PRRSV live attenuated vaccines sensitize the intravascular macrophages in such a way that promotes the production of proinflammatory cytokines on subsequent exposure to bacterial endotoxins.

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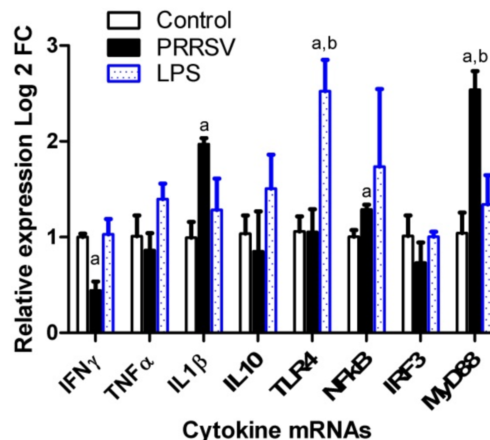

Figure 1. The expression patterns of candidate cytokine mRNAs in the PBMCs at 24 h post in vivo PRRSV vaccination and at 24 h post in vitro LPS stimulation. Values presented here are the mean ± standard error of normalized log2 expression obtained from three individual pigs. "a" indicates the significant (p<0.05) difference between contrast pairs of control vs PRRSV and control vs LPS; and "b" for PRRSV vs LPS treatments.

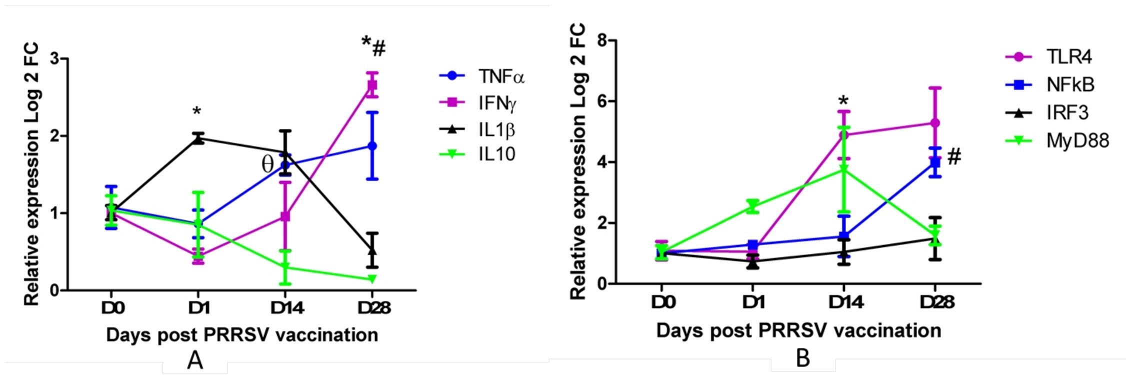

Figure 2. Temporal patterns of cytokine expression in PBMCs following PRRSV vaccination. "" indicates the significance at all time points compared with D0; "θ" and "#" for D1 vs D14 and D14 vs D28, respectively.*

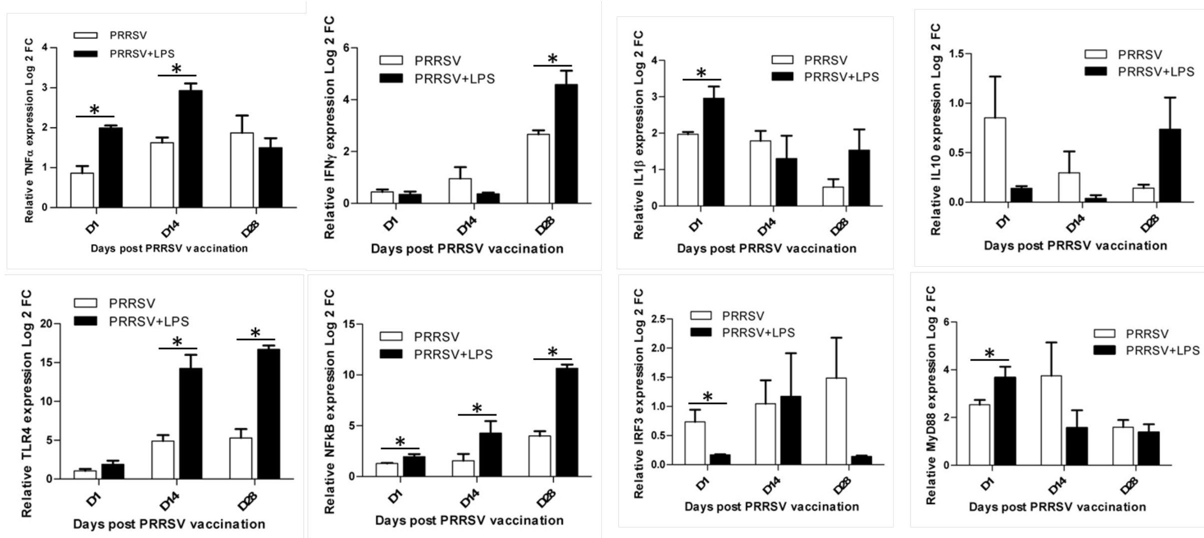

Figure 3. Expression patterns of selected cytokine mRNAs after 24 h LPS stimulation of the PBMCs collected at different time post PRRSV vaccination., "" indicates the significant difference between PRRSV vs PRRSV+LPS treatment.*