Porcine Reproductive and Respiratory Syndrome Virus Induces Interleukin-15 through the NF- κ B Signaling Pathway

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Porcine reproductive and respiratory syndrome virus (PRRSV) mainly infects macrophages/dendritic cells and modulates cytokine expression in these cells. Interleukin-15 (IL-15) is a pleiotropic cytokine involved in wide range of biological activities. It has been shown to be essential for the generation, activation, and proliferation of NK and NKT cells and for the survival and activation of CD8⁺ effector and memory T cells. In this study, we discovered that PRRSV infection upregulated IL-15 production at both the mRNA and protein levels in porcine alveolar macrophages (PAMs), blood monocyte-derived macrophages (BMo), and monocyte-derived dendritic cells (DCs). We subsequently demonstrated that the NF- κ B signaling pathway was essential for PRRSV infection-induced IL-15 production. First, addition of an NF- κ B inhibitor drastically reduced PRRSV infection-induced IL-15 production. We then found that NF- κ B was indeed activated upon PRRSV infection, as evidenced by I κ B phosphorylation and degradation. Moreover, we revealed an NF- κ B binding motif in the cloned porcine IL-15 (pIL-15) promoter, deletion of which abrogated the pIL-15 promoter activity in PRRSV-infected alveolar macrophages. In addition, we demonstrated that PRRSV nucleocapsid (N) protein had the ability to induce IL-15 production in porcine alveolar macrophage cell line CRL2843 by transient transfection, which was mediated by its multiple motifs, and it also activated NF- κ B. These data indicated that PRRSV infection-induced IL-15 production was likely through PRRSV N protein-mediated NF- κ B activation. Our findings provide new insights into the molecular mechanisms underlying the IL-15 production induced by PRRSV infection.

Porcine reproductive and respiratory syndrome (PRRS) is the most economically important infectious disease of swine industry worldwide and is characterized by respiratory disorders and pregnant sow abortion (60). PRRS is caused by porcine reproductive and respiratory syndrome virus (PRRSV), which is an enveloped, positive-strand RNA virus belonging to the family *Arteriviridae*, order *Nidovirales* (14). The PRRSV genome is about 15.4 kb in length, which has 9 open reading frames and encodes 7 structural proteins and 14 nonstructural proteins (51). The structural proteins include 2a, 2b (or E), GP3, GP4, GP5, the matrix protein (M), and the nucleocapsid (N) protein. PRRSV exhibits a highly restricted host cell tropism for the cells of the monocyte/macrophage/dendritic lineages, which play main immune functions including phagocytosis, antigen presentation, and cytokine production in innate immunity (18). Like other pathogens, PRRSV infection stimulates cytokine production, but it also has an immune suppressive activity. One of the most remarkable features of PRRSV infection is that in the lungs of pigs it fails to elicit the expression of inflammatory cytokines, particularly type I interferons (alpha/beta interferon [IFN- α / β]), interleukin-1 (IL-1), and tumor necrosis factor alpha (TNF- α), which are important in antiviral responses (54, 55). In addition, in alveolar macrophages *in vitro* and at the site of infection, PRRSV elicits only a minimal IFN- α production. At the transcriptional level, PRRSV induced the expression of IFN- β mRNA, but not IFN- α mRNA, in both alveolar macrophages and monocyte-derived dendritic cells (DCs) *in vitro* (21, 34). Similarly, PRRSV infection has been found to induce a substantially weaker, peripheral blood IFN- γ response than other viruses (39, 48, 55, 63). Likewise, PRRSV has been shown to be a poor stimulator of innate cytokine production, in contrast to most viruses, which elicit copious amounts of IFN- α and IFN- β .

IL-15 is a pleiotropic cytokine involved in a wide range of bio-

logical activities (57). It is produced by a variety of cell types including activated monocytes, macrophages, dendritic cells, epithelial cells, microglial cells, and astrocytes (8). IL-15 receptor shares the IL-2 β and γ chain (IL-15/IL-2R $\beta\gamma$) with IL-2 receptor complex, but it also has the private α chain (IL-15R α) that specifically recognizes IL-15 and allows the cytokine to carry out its own action (11, 22). IL-15R α mRNA is expressed in a variety of cells and tissues, such as liver, heart, spleen, lung, skeletal muscle, T cells, B cells, macrophages, and thymic cells (23), suggesting that the IL-15 signaling system can take actions in many tissues and cells. The role of IL-15 in host defense against viral infections is well documented, and the antiviral activity of IL-15 is primarily mediated via the activation of NK cells and NKT cells (1, 3, 6, 24). It has been shown that IL-15 is essential for the generation, activation, and proliferation of NK cells and NKT cells (28, 40). Disruption of IL-15, IL-15 receptor subunits, or IL-15 signaling components all impaired NK cell production and functions (28, 33, 37, 42). Moreover, it has been demonstrated that IL-15 is required for the maintenance and renewal of virus-specific memory and naïve CD8⁺ T cells. IL-15 regulates not only the number of the memory CD8⁺ T cells but also the activation of their functions, including IFN- γ production and cytotoxic activity, which are important to eliminate the virus (17, 65).

PRRSV induces a persistent viral infection, suggesting an ineffect-

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ficient cellular immune response. The cytotoxic function of CD8⁺ and NK cells has been shown to be downregulated even though their population is upregulated after 2 weeks of PRRSV infection (4, 15, 44, 50). Lamontagne et al. (31) also showed that NK cells and memory T cells were not significantly activated during the PRRSV infection. In contrast, Samsom et al. (49) reported that during PRRSV infection in pigs, a strong influx of NK cells and cytotoxic T cells occurred in the lungs. Interestingly, Lunney et al. (35) found that in tracheobronchial lymph nodes from PRRSV-infected pigs, IL-15 gene expression was increased. IL-15 plays an important regulatory role in both innate and adaptive immune responses during virus infection. However, there are few reported data on IL-15 in PRRSV-infected cells. Therefore, to gain a better understanding of the IL-15 response of the host to PRRSV infection, we sought to determine whether PRRSV infection induces IL-15 production in macrophage/dendritic cells and the underlying mechanisms.

In this study, we demonstrated that PRRSV induced IL-15 mRNA and protein expressions in porcine alveolar macrophages (PAMs), blood monocyte-derived macrophages (BMo), and monocyte-derived DCs. Inhibitors of protein kinase C (PKC) and NF- κ B signaling pathways considerably reduced IL-15 production in these cells. Subsequently, PRRSV N protein was proved to be involved in IL-15 production. We further cloned and identified a PRRSV-inducible region in the porcine IL-15 promoter. This region consists of IFN regulatory factor element (IRF-E) and NF- κ B motifs. Using deletion analysis, we demonstrated that the NF- κ B motif was essential for the activation of porcine IL-15 promoter by PRRSV and PRRSV N protein.

MATERIALS AND METHODS

Cells, virus, and virus preparation. Marc-145 cells are a PRRSV-permissive cell line subcloned from MA-104 cells (29), and CRL2843 cells are a porcine alveolar macrophage cell line (59). Marc-145 cells were maintained in Dulbecco's minimum essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and penicillin-streptomycin. CRL2843 cells were maintained in RPMI 1640 medium supplemented with 10% FBS and penicillin-streptomycin.

Porcine alveolar macrophages (PAMs) were obtained by postmortem lung lavage of 8-week-old specific-pathogen-free (SPF) pigs and maintained in RPMI 1640 supplemented with 10% FBS and penicillin-streptomycin. Blood monocyte-derived macrophages (BMo) were separated as previously described by Wang et al. (58). Briefly, peripheral blood was collected from SPF pigs, and peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Paque (Sigma) density gradient centrifugation following the manufacturer's procedure. PBMC were cultured in RPMI 1640 supplemented with 10% pig serum plus 25% L929 cell culture supernatant at 37°C in a humidified 5% CO₂ atmosphere. After 2 h of incubation, cells were washed with RPMI 1640 to remove nonadherent cells; the adherent cells were the enriched BMo. To get mature porcine monocyte-derived DCs, monocytes from PBMC were cultured in RPMI 1640 medium supplemented with 10% FBS, recombinant porcine granulocyte-macrophage colony-stimulating factor (rpGM-CSF) (100 ng/ml) (R&D Systems), and recombinant porcine interleukin-4 (rpIL-4) (100 ng/ml) (R&D Systems). At day 6, lipopolysaccharide (LPS) was added (1 μ g/ml) (Sigma), and the cells were cultured for another 24 h (13).

PRRSV strains CH-1a (the first type 2 PRRSV strain isolated in China), VR2332 (the prototypical North American type 2 isolate), AV (a type 2 isolate from China), and HV (a highly pathogenic [HP]-PRRSV isolate) were propagated in PAMs. Virus preparations were titrated, and then stored at -80°C.

Reagents. NF- κ B inhibitor (BAY11-7082), PKC inhibitor (GF-109203X), MEK inhibitor (PD98059), p38 inhibitor (SB203580), and

TABLE 1 Sequences of the primers used for PRRSV structural genes (from PRRSV strain CH-1a) cloning and N protein deletion mutants

Name	Sequence
ORF2 forward	5'-GGGGTACCATGAAATGGGGTCTATG-3'
ORF2 reverse	5'-CGGGATCCTCACCGTGAGTTCAAA-3'
ORF3 forward	5'-GGGGTACCATGGCTAATAGCTGTAC-3'
ORF3 reverse	5'-CGGGATCCTATCGCCGTGCGGCAC-3'
ORF4 forward	5'-GGGGTACCATGGCTGCGCCCTTC-3'
ORF4 reverse	5'-CGGGATCCTCAAATGGCCAGTAGG-3'
ORF5 forward	5'-GGGGTACCATGTTGGGGAAATGCT-3'
ORF5 reverse	5'-CGGGATCCTAGAGACGACCCCAT-3'
ORF6 forward	5'-GGGGTACCATGGGGTCGTCTAG-3'
ORF6 reverse	5'-CGGGATCCTTATTGGCATATTTG-3'
C forward ^a	5'-CGCGGTACCATGCCAAATAACAACGGCAAG-3'
C85 reverse	5'-CGCGGATCCTCACTGGTTAAAGGCACTCTG-3'
C50 reverse	5'-CGCGGATCCTCACGGGCTTTTCTTCTTATT-3'
C43 reverse	5'-CGCGGATCCTTACTTCCCGGTCCCTTG-3'
C25 reverse	5'-CGCGGATCCTTACATCTGGCACAGCTGATTG-3'
N18 forward	5'-CGGGGTACCATGCCAGTCAATCAGCTGTGC-3'
N29 forward	5'-CGGGGTACCATGATCATTGCCCAACAA-3'
N40 forward	5'-CGGGGTACCATGGGACCGGGGAAGAAAAAT-3'
N52 forward	5'-CGGGGTACCATGAAGCCCCATTTTCTCTAG-3'
N69 forward	5'-CGGGGTACCATGCCTAGTGAGCGGCAATTG-3'
N reverse ^b	5'-CGCGGATCCTCATGCTGAGGGTGATGC-3'

^a The forward primer of C85, C50, C43, and C25 N protein deletion mutants.

^b The reverse primer of N18, N29, N40, N52, and N69 N protein deletion mutants.

phosphatidylinositol 3-kinase (PI3K) inhibitor (LY294002) were purchased from Enzo Life Sciences. An enzyme-linked immunosorbent assay (ELISA) kit for porcine IL-15 was purchased from R&D Systems. TRIzol was from Invitrogen Life Technology. RNase-Free DNase and the Dual-Glo luciferase assay system were purchased from Promega. Perfect Real Time SYBRPremix *Ex Taq* was from TaKaRa.

IL-15 mRNA quantification. PAMs, BMo, and DCs were infected with PRRSV at a multiplicity of infection (MOI) of 1.0 for 6, 12, or 24 h. Total RNA was extracted from cells using TRIzol reagent (Invitrogen), and 1 μ g of RNA was used for cDNA synthesis using Moloney murine leukemia virus (MMLV; Promega). Real-time PCR was performed using specific primers for porcine IL-15 (forward 5'-GCTCATCCCAATTGCAAGT-3', reverse 5'-TTCCTCCAGCTCCTCACATT-3') and the Real Time SYBR master mix kit (TaKaRa) by following the manufacturer's instructions to the ABI7500 real-time PCR system. The IL-15 gene expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (forward 5'-CCTTCCGTCCTCCTACGCAAC-3', reverse 5'-GACGCCTGCTTCACCACCTTCT-3') and presented as fold induction relative to medium control.

ELISA for IL-15. The levels of secreted IL-15 were measured in cell culture supernatants using commercially available porcine IL-15 ELISA kits (R&D Systems) according to the manufacturer's instructions.

Construction of PRRSV structural protein gene expression vectors and nucleocapsid protein deletion mutants. PRRSV structural protein genes including ORF2, ORF3, ORF4, ORF5, ORF6, and ORF7 (N) of PRRSV strain CH-1a were amplified by PCR using specific primers, and the PCR products were subcloned into the pcDNA3.1⁺ vector. All construction vectors were confirmed by sequencing (compared with PRRSV strain CH-1a sequence, GenBank accession number [AY032626.1](#)). The PRRSV N protein deletion mutants were constructed using specific primers, and all of the deletion mutants' products were subcloned into the pcDNA3.1⁺ vector. All the primers are listed in [Table 1](#).

Construction of the porcine IL-15 promoters. Genomic DNA was extracted and purified from the porcine liver by the phenol-chloroform method. The fragment flanking the porcine IL-15 gene 5' sequence was obtained by using a genomic walking kit (TaKaRa, Japan) after three rounds of thermometric asymmetry PCR assays using primers SP1 (5'-T

AACTTGCTCTTCCCGTTTCAGG-3'), SP2 (5'-AAACATAACCTTC TCTCCATCTCC-3'), and SP3 (5'-CCCCACACCCAGTCTTTCTCAAT A-3') following the manufacturer's procedure. The primers SP1 to SP3 were based on the *Sus scrofa* IL-15 gene (NM_214390.1). The fragment was subcloned into the pMD18-T vector, and the nucleotide sequence was determined by DNA sequencing. The primers designed for the promoter/luciferase construction below were based on the cloned fragment.

The 1,100-bp IL-15 promoter sequence from nucleotides (nt) -1023 to +77 relative to the translation initiation site (+1) was inserted into the luciferase reporter vector pGL3-basic (Promega) at the BglII site to create the IL-15 promoter (-1023/77-luc). The IL-15 promoter deletion mutants were generated by PCR using the forward primers 5'-GCCCCCGG GGGAATATGGGTAATATACCAATAAGC-3' (for -579/77-luc), 5'-TCCCCCGGGCATCATAGGGCTGTAAATGCTAAAC-3' (for -372/77-luc), and 5'-TCCCCCGGGGTAAACCCCTGAACACTTCAATTT-3' (for -172/77-luc), and the reverse primer was 5'-GATCGCAGATCTTT AGAAGAAGAG-3'. The NF- κ B, IRF-E, and NF- κ B/IRF-E element deletion IL-15 promoters including -1023/77(Δ NF- κ B)-luc, -372/77(Δ NF- κ B)-luc, -372/77(Δ IRF-E)-luc, and -372/77(Δ NF- κ B, Δ IRF-E)-luc were generated by overlapping PCR from the -1023/77-luc or -372/77 promoter pGL3 vector.

Inhibition of signal transduction pathways. PAMs were pretreated with dimethyl sulfoxide (DMSO), PI3K inhibitor LY294002 (5 μ M), MEK inhibitor PD98059 (10 μ M), p38 mitogen-activated protein kinase (MAPK) inhibitor SB203580 (5 μ M), PKC inhibitor GF-109203X (1 μ M), or NF- κ B inhibitor BAY11-7082 (1 μ M) for 1 h and then infected with PRRSV at an MOI of 1 in the presence of the inhibitors. Twenty-four hours later, supernatants were harvested for secreted IL-15 by ELISA and cells were harvested for IL-15 mRNA analysis by real-time PCR. Marc-145 cells seeded on a 12-well plate were transfected using Lipofectamine LTX and Plus Reagent (Invitrogen) with IL-15 -373/77 or pGL3-luc luciferase vector (1 μ g), and 24 h later cells were infected with PRRSV at an MOI of 3 for 24 h in the presence or absence of the NF- κ B inhibitor BAY11-7082 (1 μ M). CRL2843 cells seeded on a 12-well plate were pretreated with NF- κ B inhibitor BAY11-7082 (1 μ M) for 1 h and then transfected using Lipofectamine 2000 reagent (Invitrogen) with IL-15 -373/77-luc or pGL3-luc luciferase vector alone or plus PRRSV nucleoprotein (N) vector (1 μ g for each plasmid) in the presence or absence of the inhibitors for 48 h. Cells were harvested for luciferase analysis.

Western blot analysis. Marc-145 cells and CRL2843 cells were either inoculated with PRRSV (MOI = 1.0) for 30 min, 3 h, 6 h, 12 h, or 24 h; transfected with different amounts of PRRSV N protein vectors for 24 h; or stimulated with poly(I:C) for 3 h. Whole-cell extracts were prepared by being lysed in NP-40 lysis buffer supplemented with protease and phosphatase inhibitors. Similar amounts of protein from each extract were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes. After blocking, the membranes were incubated for 2 h at room temperature with the following primary antibodies as indicated: anti-I κ B- α (1:1,000; Cell Signaling Technology), anti-p-I κ B- α (1:1,000; Cell Signaling Technology), anti-PRRSV N protein SDOW17 (1:2,000; Rural Technologies), and anti- β -actin (1:5,000; Sigma, St. Louis, MO). The membranes were then incubated with the appropriate secondary antibody for 1 h (1:5,000). The antibodies were visualized by use of the ECL reagent according to the manufacturer's instructions.

Confocal microscopy. CRL2843 cells were transfected with 500 ng of N protein vector or pcDNA3.1 vector control and then fixed with 4% paraformaldehyde 24 h later. Cells were permeabilized with 0.1% Triton X-100, washed 3 times, and then stained with the rabbit polyclonal anti-NF- κ B p65 antibody (1:100; Cell Signaling Technology) and mouse monoclonal antibody anti-PRRSV N protein SDOW17 (1:10,000; Rural Technologies). NF- κ B p65 staining and PRRSV N protein staining were visualized with anti-rabbit IgG secondary antibody conjugated with fluorescein isothiocyanate (FITC) (1:100; Sigma) and anti-mouse IgG secondary antibody conjugated with tetramethyl rhodamine isothiocyanate

(TRITC) (1:100; Sigma). Nuclei were stained with DAPI (4',6-diamidino-2-phenylindole). Immunofluorescence was observed using equipment from Leica Microsystems CMS GmbH.

Luciferase reporter assay. Marc-145 cells seeded on a 12-well plate were transfected using Lipofectamine LTX and Plus Reagent (Invitrogen) with IL-15 -373/77-luc, -373/77(Δ NF- κ B)-luc, -373/77(Δ IRF)-luc, -373/77(Δ NF- κ B, Δ IRF)-luc, or pGL3-luc luciferase vector (1 μ g for each plasmid), and 24 h later cells were mock infected or infected with PRRSV at an MOI of 3. Cells were then harvested for luciferase activity analysis 24 h later. CRL2843 cells seeded on a 12-well plate were transfected using Lipofectamine 2000 reagent (Invitrogen) with pIL-15 -1023/77-luc, -1023/77(Δ NF- κ B)-luc, -373/77-luc, -373/77(Δ NF- κ B)-luc, -373/77(Δ IRF)-luc, -373/77(Δ NF- κ B, Δ IRF)-luc, or pGL3-luc luciferase vector (1 μ g for each plasmid) alone or plus PRRSV N protein expression vector (1 μ g for each plasmid). Forty-eight hours later, cells were harvested for luciferase activity analysis.

To determine the essential motif for PRRSV N protein to activate the IL-15 promoter, CRL2843 cells seeded on a 12-well plate were transfected either with pIL-15 -1023/77-luc alone, with -373/77-luc alone or cotransfected with pIL-15 -1023/77-luc, or with -373/77-luc with different PRRSV N protein deletion mutants or control vector. Cells were then harvested 48 h later for luciferase activity analysis.

Luciferase assays were performed with extracts prepared using a dual luciferase reporter assay system according to the manufacturer's instructions (Promega).

Statistical analysis. All experiments were performed with at least three independent replicates. Statistical analysis was performed using GraphPad Prism software, and differences in data were evaluated by Student's *t* test. A *P* value of <0.05 was considered to be statistically significant.

RESULTS

PRRSV upregulates IL-15 production in PAMs, BMo, and DCs.

To investigate whether PRRSV can induce IL-15 production, we infected several PRRSV-susceptible cells with different strains of PRRSV, including porcine alveolar macrophages, blood monocyte-derived macrophages, and blood monocyte-derived dendritic cells, and then measured the mRNA and protein levels of IL-15. Our results showed that the PRRSV strain CH-1a significantly induced IL-15 production both at the mRNA level and at the protein level in PAMs at 6, 12, and 24 h (Fig. 1A and B), in BMo (Fig. 1C and D) between 6 and 24 h, and in dendritic cells (Fig. 1E and F) at 12 and 24 h postinfection. Similar results were also obtained with other strains such as VR2332 (the prototypical North American type 2 isolate), AV (a type 2 isolate from China), and HV (an HP-PRRSV isolate) (data not shown), indicating that PRRSV-induced IL-15 production was not strain dependent. However, IL-15 induction by strain HV was postponed until 36 h postinfection (data not shown), indicating that a difference with respect to inducing IL-15 exists between the HP-PRRSV strain and the type 2 American strain. The induction of IL-15 by PRRSV was likely replication dependent because compared with that of live PRRSV, the UV-inactivated PRRSV-induced IL-15 production was significantly reduced both at the mRNA level and at the protein level (Fig. 1A and B). PRRSV strain CH-1a was used in the following studies.

Cloning porcine IL-15 promoter and mapping an essential PRRSV-responsive region. To study the mechanisms underlying the transcriptional regulation of PRRSV-induced IL-15 production, a 1,100-bp fragment of the 5'-flanking region of porcine IL-15 gene was cloned using the genome walking kit (Fig. 2A). One hundred percent homology was found between the cloned gene and the reference sequences in NCBI (NC_010450.3; *Sus*

FIG 1 IL-15 is upregulated in PRRSV-infected porcine alveolar macrophages (PAMs), blood monocyte-derived macrophages (BMo), and blood monocyte-derived dendritic cells (DCs). (A and B) PAMs were inoculated with medium alone, PRRSV (CH-1a strain), or heat-inactivated PRRSV at a multiplicity of infection (MOI) of 1.0. (C and D) BMo were inoculated with medium alone or PRRSV at an MOI of 1.0. (E and F) DCs were inoculated with medium alone or PRRSV at an MOI of 1.0. Total RNA was extracted from cell lysates at 6, 12, and 24 h postinoculation. IL-15 mRNA was quantified by real-time PCR (A, C, and E), and results were normalized to GAPDH and expressed as fold induction over medium alone at 6 h postinoculation. Supernatants were harvested at 6, 12, and 24 h postinoculation, and levels of IL-15 (pg/ml) released were determined by ELISA (B, D, F). Mean $\pm$ standard error of the mean (SEM) from three independent experiments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$ (for live virus compared to medium control); #, $P < 0.05$; ##, $P < 0.01$ (for live PRRSV compared to UV inactivated). Statistical analysis was performed by Student's t test.

scrofa). Using the bioinformatics approach (<http://www.cbrc.jp/research/db/TFSEARCH.html>), several putative transcriptional regulatory elements were found in the 5'-flanking region of the pIL-15 gene, including AP-2 (−941 to −936, −925 to −920, −909 to −904), Sp1 (−895 to −890), CCAAT-box (−720 to −716, −56 to −52), IRF-E (−256 to −242), and NF- κ B (−240 to −231). IRF-E and NF- κ B are separated by 1 nucleotide.

To evaluate the porcine IL-15 promoter activity and determine the region that responds to PRRSV infection, serial deletions starting from the 5' end of the promoter were generated and are schematically shown in Fig. 2B. These constructs were transfected into Marc-145 cells, and the luciferase activities were assessed with or without PRRSV infection. Our results showed that all the deletion constructs demonstrated higher basal-level luciferase activity than

the full-length construct, with the construct −172/77-luc being the highest. This observation suggests that there may be a regulatory motif(s) in the 5' region of the cloned IL-15 promoter that downregulates its transcription. After PRRSV infection, all the promoter constructs showed significantly higher luciferase activities, among which the construct −372/77-luc promoter was much more efficiently stimulated by PRRSV infection than the others (Fig. 2C), which manifested a 2-fold induction over its basal-level activity. Therefore, we used IL-15 promoter construct −372/77-luc, which contains an IRF-E motif and an NF- κ B motif, in the following studies.

PRRSV N protein induces IL-15 production. To examine which PRRSV protein can induce IL-15 production, CRL2843 cells were cotransfected with −372/77-luc promoter plasmid and

FIG 2 Cloning, sequence analysis, and characterization of porcine IL-15 promoter. (A) Cloning and sequence analysis of the 1,100-bp porcine IL-15 gene 5'-flanking region. The putative transcription factor binding sites are shown in boxes; positions of the putative regulatory motifs relative to the translation initiation site ATG are shown (denoted as +1). (B) Schematic representation of the porcine IL-15 promoter and promoter deletion mutants inserted into pGL3 basic luciferase vectors: -1023/77-luc, -579/77-luc, -372/77-luc, and -172/77-luc porcine IL-15 promoter vectors. The relative lengths and positions of the 5'-ends of these fragments are indicated. (C) The -1023/77-luc, -579/77-luc, -372/77-luc, -172/77-luc porcine IL-15 promoter vectors and pGL3 empty vector were transfected into Marc-145 cells. Twenty-four hours later, cells were inoculated with PRRSV (MOI = 3.0) or medium; cells were harvested to determine luciferase activity 24 h postinfection. Data are mean $\pm$ SEM from three independent experiments. *, $P < 0.05$; **, $P < 0.01$; as determined by Student's t test.

each of the PRRSV structural protein expression vectors. The cells were harvested 48 h later, and luciferase activity was measured. Our results showed that only PRRSV N protein (ORF7) significantly enhanced IL-15 promoter activity (Fig. 3A). To further examine whether PRRSV structural proteins can induce IL-15 production, we transfected CRL2843 cells with the plasmids containing each of the PRRSV structural protein genes. Cells and supernatant were harvested 24 h later, and IL-15 production was measured by real-time PCR (Fig. 3B) and ELISA (Fig. 3C). Consistent with above luciferase activity data, PRRSV N protein (ORF7) significantly induced IL-15 production with a 4.5-fold increase in its mRNA expression and a 3-fold increase in the protein levels compared to control. Thus, we conclude that PRRSV N protein can induce IL-15 production. We also examined the ability of PRRSV nonstructural proteins to induce IL-15 production in CRL2843 cells. However, none of the PRRSV nonstructural proteins can increase IL-15 gene expression (data not shown).

The middle region of PRRSV N protein is involved in pIL-15 activation. PRRSV N protein contains two nuclear localization signal (NLS) sequences located at amino acids 10 to 13 (NLS-1) and 41 to 47 (NLS-2), a nucleolar localization signal (NoLS) sequence at amino acids 41 to 72, and an N-N noncovalent interaction domain at amino acids 30 to 37 (46, 47, 62). To determine which domain of PRRSV N protein is essential for pIL-15 activation, nine N protein deletion mutants were constructed. The truncated mutants included C85 (1 to 85 aa), C50 (1 to 50 aa), C43 (1 to 43 aa), C25 (1 to 25 aa), N18 (18 to 123 aa), N29 (29 to 123 aa), N40 (40 to 123 aa), N52 (52 to 123 aa), and N69 (69 to 123 aa)

(Fig. 4A). Each of the mutant vectors was cotransfected into CRL2843 cells with constructs encoding pIL-15 full-length promoter -1023/77-luc (Fig. 4B). The luciferase activities were measured 48 h later. Our results showed that C85, N18, and N29, which contain the intact N-N noncovalent interaction domain and the NLS-2 and NoLS domains but without C terminal, N terminals, and NLS-1, could induce pIL-15 promoter luciferase activity as efficiently as the intact PRRSV N protein. C50, which contains the intact N-N noncovalent interaction and NLS-2 domains, also significantly activated the pIL-15 promoter. However, compared to that of C85, N18, and N29, its ability to activate the pIL-15 promoter was significantly decreased, suggesting that NoLS 41 to 72 may play a role in PRRSV N protein's activation of the IL-15 promoter. In contrast, N40, which contains the intact NLS-2 and NoLS domains but not the N-N noncovalent interaction domain, could not induce IL-15 promoter activity. C43, which lacks NoLS and NLS-2, could not activate the IL-15 promoter. Similar results were obtained when the pIL-15 partial promoter -372/77-Luc was used in the above experiments (Fig. 4C). We also found that the ability of PRRSV N protein as well as of its mutants to activate the pIL-15 -372/77-luc promoter was stronger than that of the full-length promoter (-1023/77-luc) (about 2.5-fold versus 2.0-fold over the control vector). This observation reinforced the presumption that the pIL-15 full-length promoter contains a negative regulatory element at the region of -1023/-377. Altogether, these data suggested that NLS 41 to 47, N-N noncovalent interaction, and NoLS domains of N protein were essential for pIL-15 promoter activation and that the lack of any of

FIG 3 PRRSV N protein induces IL-15 production. (A) PRRSV structural protein expression vectors, including ORF2, ORF3, ORF4, ORF5, ORF6, ORF7, and pcDNA 3.1 vector control, were cotransfected into CRL2843 with pIL-15 -372/77 promoter pGL3 vector. Forty-eight hours later, cells were harvested for luciferase activity detection. Poly(I-C) treatment was set up as the positive control. (B and C) PRRSV structural protein expression vectors were transfected into CRL2843 cells. Twenty-four hours later, total RNA was extracted from cell lysates. IL-15 mRNA was quantified by real-time PCR (B), and results were normalized to GAPDH and expressed as fold induction over pcDNA vector control. (C) Supernatants were harvested 24 h after transfection, and levels of IL-15 (pg/ml) released were determined by ELISA. Data are mean $\pm$ SEM from three independent experiments. *, $P < 0.05$; **, $P < 0.01$; as determined by Student's t test.

these domains could lead to the decrease of pIL-15 promoter activation.

The NF- κ B and PKC signaling pathways are involved in PRRSV-induced pIL-15 upregulation. To dissect the signaling pathways involved in PRRSV-induced IL-15 production, PAMs were pretreated for 1 h with the inhibitors of the key signaling molecules including NF- κ B, MEK, p38 MAPK, PI3K, or PKC before PRRSV infection. Twenty-four hours later, the expressions of

FIG 4 PRRSV N protein middle region involved in pIL-15 activation. (A) Schematic diagram represents the PRRSV N protein serial deletion mutant constructs. (B and C) The pIL-15 -1023/77 promoter (B) and the pIL-15 -372/77 promoter (C) were cotransfected into CRL2843 cells with PRRSV N protein deletion mutant vectors or pcDNA3.1 empty vector. Forty-eight hours later, cells were harvested for luciferase activity detection. Poly(I-C) treatment for 8 h was set up as positive control; the pGL3 empty vector cotransfected with pcDNA3.1⁺ vector was the negative control. Data are mean $\pm$ SEM from three independent experiments. *, $P < 0.05$; **, $P < 0.01$; as determined by Student's t test.

IL-15 were analyzed by real-time PCR (Fig. 5A) and ELISA (Fig. 5B). As shown in Fig. 5A and B, GF-109203X (PKC inhibitor) and BAY11-7082 (NF- κ B inhibitor) significantly inhibited the induction of IL-15 gene expression by PRRSV infection. Inhibition of MEK (PD98059) and PI3K (LY294002) slightly increased IL-15 mRNA expression, but there was no effect on IL-15 secretion. MAPK inhibitor SB202190 had no effect on PRRSV-induced IL-15 production. These findings suggest that the PKC and NF- κ B pathways may be involved in the PRRSV-induced IL-15 production. To confirm that NF- κ B pathways are indeed involved in PRRSV-induced IL-15 production, Marc-145 cells were pretreated with BAY11-7082 (NF- κ B inhibitor) for 1 h and then

FIG 5 Effects of signal transduction inhibitors on IL-15 expression induced by PRRSV infection or PRRSV N protein. (A and B) PAMs were pretreated with inhibitors of PKC (GF-109203X [GF]), NF- κ B (BAY11-7082 [BAY]), MEK (PD98059 [PD]), PI3K (LY294002 [LY]), MAPK (SB202190 [SB]), or DMSO control for 1 h, and then cells were inoculated with or without PRRSV (MOI = 1.0). Twenty-four hours later, total RNAs were extracted for detecting IL-15 mRNA by real-time PCR (A), and cell culture supernatants were harvested for testing IL-15 protein by ELISA (B). (C) Marc-145 cells were pretreated with NF- κ B inhibitor (BAY11-7082 [BAY]) or DMSO control for 1 h and then were transfected with pIL-15 -372/77-luc pGL3 vector or pGL3-luc basic control vector. Twenty-four hours later, cells were infected with PRRSV and cells were harvested for luciferase activity detection 24 h after PRRSV infection. (D) CRL2843 cells were pretreated with NF- κ B inhibitor (BAY11-7082 [BAY]) or medium control for 1 h. Then, pIL-15 -372/77-luc pGL3 vector or pGL3-luc basic control vector was cotransfected with PRRSV N expression vector into CRL2843 cells. Cells were harvested for luciferase activity detection 48 h later. Data are mean $\pm$ SEM from three independent experiments. Inhibitors were present at the end of the experiments. *, $P < 0.05$; as determined by Student's t test.

transfected with pIL-15 promoter -372/77-luc. Twenty-four hours later, the cells were inoculated with PRRSV in the presence or absence of the inhibitor, and the IL-15 promoter luciferase activity was measured 24 h postinfection. As shown in Fig. 5C, BAY11-7082 significantly suppressed PRRSV-induced IL-15 luciferase activity (about 50% decrease), confirming that the NF- κ B pathway is involved in IL-15 production induced by PRRSV infection.

To investigate whether the NF- κ B pathway is also involved in PRRSV N protein-induced IL-15 production, CRL2843 cells were cotransfected with pIL-15 promoter -372/77-luc and PRRSV N protein in the presence or absence of BAY11-7082. Our data showed that NF- κ B inhibitor significantly decreased IL-15 promoter luciferase activity (Fig. 5D), suggesting that the NF- κ B pathway may be involved in PRRSV N protein-induced IL-15 production.

PRRSV and PRRSV N protein activate NF- κ B. It is well known that the activation of NF- κ B requires phosphorylation-mediated I κ B degradation (27). We therefore examined whether I κ B is phosphorylated and degraded during the course of PRRSV infection. PAMs infected with PRRSV for different time periods (0.5, 3, 6, 12, and 24 h) were collected for Western analysis using a phospho-specific antibody for p-I κ B- α and an antibody for I κ B.

As expected, I κ B- α phosphorylation started to increase at 30 min postinfection and continued increasing through the end of the experiment 24 h postinfection. Conversely, the I κ B protein level started to decrease 30 min post-PRRSV infection and kept decreasing with the increase of infection duration (Fig. 6A).

To investigate whether PRRSV N protein also induced I κ B phosphorylation, CRL2843 cells were transfected with different amounts of PRRSV N protein (50 ng, 100 ng, 200 ng, 500 ng, and 1,000 ng). Twenty-four hours later, the cells were harvested and analyzed by Western blotting. As shown in Fig. 6B, I κ B- α phosphorylation was undetectable in control cells, but after transfection of PRRSV N protein, it had increased as the amount of N protein had increased. On the contrary, I κ B degradation which correlated with the increase in I κ B- α phosphorylation was observed. To further confirm that PRRSV N protein can activate NF- κ B, we evaluated the effect of PRRSV N protein on NF- κ B subunit p65 translocation to the nucleus. Our results showed that in untransfected CRL2843 cells, p65 (green) mainly existed in cytoplasm (Fig. 6C). However, in PRRSV N protein vector-transfected cells, nuclear accumulation of endogenous p65 was observed (Fig. 6D and E). Although the PRRSV N protein had the nuclear localization signal (shown as red in Fig. 6D and E), it

FIG 6 Activation of NF- κ B signaling pathway by PRRSV and PRRSV N protein. (A) PAMs were inoculated with PRRSV (MOI = 1.0), and cells were harvested at 0.5, 3, 6, 12, and 24 h postinfection. (B) CRL2843 cells were transfected with different amounts of N protein expression vector, and cells were harvested 24 h later. Cells were treated with poly(I:C) for 3 h as a positive control. Harvested cells were lysed for Western blotting to examine levels of I κ B- α , p-I κ B- α , N protein, and β -actin. (C to E) NF- κ B p65 and PRRSV N protein localizations were determined by confocal microscopy. CRL2843 cells were transfected with pcDNA3.1 control vector (C) or transfected with PRRSV N protein expression vector (D and E) for 24 h. NF- κ B p65 (green) and PRRSV N protein (red) were then detected by indirect immunofluorescence staining by antibodies against p65 and PRRSV N protein. Nucleus (blue) was stained with DAPI.

distributed both in cytoplasm and nucleus, and in both cases p65 was able to translocate to the nucleus. Taking these data together, we conclude that PRRSV N protein could activate NF- κ B pathway indirectly.

NF- κ B binding element in the pIL-15 promoter is essential for IL-15 production. Based on sequence analysis, the pIL-15 promoter has been shown to have several regulatory motifs including NF- κ B and IRF-E binding sites, which are important for its activation by other stimuli (7, 8). We have mapped the promoter region responding to PRRSV infection to -372/77, which contains the binding elements for NF- κ B and IRF-E. To further test whether both binding elements are essential for PRRSV-induced IL-15 production, we deleted either the NF- κ B or the IRF-E binding element or both from pIL-15 -372/77 and designated these mutants as -372/77(Δ NF- κ B)-luc, -372/77(Δ IRF-E)-luc, and -372/77(Δ NF- κ B, Δ IRF-E)-luc, which are schematically shown in Fig. 7A. Next, we transfected Marc-145 cells with each of the IL-15 promoter constructs, and 24 h later cells were infected with or without PRRSV. Cells were harvested 24 h postinfection, and luciferase activity was examined. As shown in Fig. 7B, when the NF- κ B motif was deleted, the IL-15 luciferase promoter [-372/77(Δ NF- κ B)-luc] lost its ability to respond to PRRSV stimulation and showed a 6-fold decrease in luciferase activity compared with the intact -372/77-luc. In contrast, without the IRF-E motif, the luciferase activity of the IL-15 luciferase promoter [-372/77(Δ IRF-E)-luc] responding to PRRSV infection was only slightly affected compared to that of the intact -372/77-luc, suggesting that the IRF-E motif is not essential for PRRSV-induced IL-15 production. However, when both NF- κ B and IRF-E binding ele-

ments were deleted, the ability of the IL-15 luciferase promoter to respond to PRRSV infection was fully abolished. Therefore, we conclude that the deletion of NF- κ B impairs PRRSV-induced IL-15 production.

To determine the role of NF- κ B motif in IL-15 promoter activation by PRRSV N protein, the -372/77 deletion mutants were cotransfected with PRRSV N expression vector into CRL2843 cells. Cells were harvested and measured for luciferase activity 48 h later. Similarly, without the NF- κ B motif, the luciferase activity of the IL-15 luciferase promoter was fully repressed (9-fold decrease) compared with that of intact -372/77-luc (Fig. 7C). We also showed that the NF- κ B motif was essential for the full-length IL-15 promoter (-1023/77-luc) activation by PRRSV N protein, deletion of which [-1023/77(Δ NF- κ B)-luc] totally abrogated IL-15 promoter luciferase activity (Fig. 7D). Hence, the NF- κ B motif is critical for PRRSV N protein to activate the IL-15 promoter. Taking these data together, we conclude that the NF- κ B motif is essential for PRRSV-induced IL-15 production.

DISCUSSION

In this study, we examined the ability of PRRSV, as well as PRRSV proteins, to induce IL-15 expression. We showed that PRRSV induced IL-15 mRNA and protein expressions in porcine alveolar macrophages, blood monocyte-derived macrophages, and monocyte-derived dendritic cells. The addition of inhibitors of PKC and NF- κ B signaling pathways considerably reduced IL-15 production in these cells, suggesting that PKC and NF- κ B signaling pathways were involved in IL-15 induction by PRRSV infection. We further showed that PRRSV N protein could induce IL-15 in por-

FIG 7 NF- κ B binding motif in the pIL-15 $-372/77$ promoter region is essential for IL-15 production. (A) Schematic diagram represents pIL-15 promoter deletion mutant constructs. (B) Marc-145 cells were transfected with pIL-15 $-372/77$ promoter, $-372/77(\Delta$ NF- κ B)-luc, $-372/77(\Delta$ IRF-E)-luc, $-372/77(\Delta$ NF- κ B/ Δ IRF-E)-luc, or pGL3 empty vector. Twenty-four hours later, cells were inoculated with PRRSV (MOI = 3.0) or medium control and then harvested for luciferase activity detection 24 h postinfection. (C) pIL-15 $-372/77$ promoter, $-372/77(\Delta$ NF- κ B)-luc, $-372/77(\Delta$ IRF-E)-luc, $-372/77(\Delta$ NF- κ B/ Δ IRF-E)-luc, or pGL3 basic control vector was cotransfected into CRL2843 cells with PRRSV N protein or pcDNA3.1 control. Cells were harvested for luciferase activity detection 48 h later. (D) pIL-15 $-1023/77$ promoter, $-1023/77(\Delta$ NF- κ B)-luc, or pGL3 basic control vector was cotransfected into CRL2843 cells with PRRSV N protein or pcDNA3.1 control. Cells were harvested for luciferase activity detection 48 h later. Data are mean $\pm$ SEM from three independent experiments. *, $P < 0.05$; as determined by Student's t test.

cine alveolar macrophage cell line CRL2843 cells. Furthermore, porcine IL-15 promoter was cloned, and we found that the region responsive to PRRSV infection contains the binding motifs for IRF-E and NF- κ B. Using deletion analysis, we demonstrated that the NF- κ B binding motif was essential for the activation of porcine IL-15 promoter by PRRSV and PRRSV N protein.

It has been reported that many viruses such as influenza virus, Epstein-Barr virus (EBV), reovirus, respiratory syncytial virus (RSV), herpes simplex virus 1 (HSV-1), vesicular stomatitis virus (VSV), and Sendai virus can upregulate IL-15 production (3, 19, 20, 32). In the present study, we found that PRRSV infection could also induce IL-15 production in macrophages/dendritic cells *in vitro*. This upregulation was detectable as early as 6 h postinfection and persisted for many hours. IL-15 release was reduced significantly by UV inactivation, suggesting that the induction of IL-15 by PRRSV may be largely replication dependent. As virus replication occurs, accumulation of virus proteins and double-stranded RNA (dsRNA) increases, and then the host cells could sense them and become activated. Indeed, some virus-induced cytokines do require viral replication and cell cycle proteins for their production (5, 12, 32). UV-inactivated PRRSV particles slightly induced IL-15 mRNA expression at 6 h postinfection (Fig. 1A), and IL-15 release was significantly increased at 24 h postinfection (even though significantly low compared to what was seen for live virus particles) (Fig. 1B), implicating that PRRSV proteins might have the ability to induce IL-15 production. Actually, we found that NF- κ B could be activated 30 min after PRRSV inoculation (Fig. 6A), and it was believed that this activation was independent of virus replication. We also examined the ability of different type 2 PRRSV strains to induce IL-15 production in macrophages and

found no difference among the conventional type 2 strains we tested. Interestingly, however, the HP-PRRSV strain HV-induced IL-15 production was dramatically delayed, as IL-15 mRNA expression was not increased until 36 h postinfection (unpublished data), suggesting that there is a difference in IL-15 induction between the conventional type 2 strain and the HP-PRRSV strain. The implication of this difference in PRRSV pathogenesis needs to be investigated further.

We then investigated the ability of PRRSV proteins to induce IL-15 production in CRL2843 cells (porcine macrophage cell line). We found that PRRSV N protein could activate pIL-15 promoter activity and induce IL-15 expression at the mRNA and protein levels. PRRSV N protein is the most abundant viral protein in the virion (51). It is highly immunogenic in pigs and believed to play some role in cell-mediated immune response against PRRSV infection (10, 16, 26, 41, 45, 64). However, it is hard to predict what role PRRSV N protein plays by inducing IL-15 production in PRRSV pathogenesis. In this study, we used poly(I:C) (analog of dsRNA) as a positive control, and it could induce IL-15 production in CRL2843 cells, suggesting that CRL2843 cells could recognize viral dsRNA generated during PRRSV replication and produce IL-15. Therefore, PRRSV infection-induced IL-15 expression could be via both PRRSV N protein and viral dsRNAs generated during replication.

Treatment of porcine alveolar macrophages with NF- κ B inhibitor prior to infection with PRRSV significantly reduced IL-15 production compared with what was seen for cells infected by PRRSV only, suggesting that the NF- κ B signaling pathway is involved in the production of IL-15. Many viruses such as human T-cell leukemia virus 1 (HTLV-1), RSV, and Newcastle disease

virus (NDV) induce IL-15 production through the activation of the NF- κ B pathway (7, 8, 19). NF- κ B defines a family of dimeric transcription factors, and the predominant species of the NF- κ B family is the p50–p65 complex, which is retained in the cytoplasm by its inhibitor I κ B (27). NF- κ B is a heterodimeric transcription factor involved in the activation of a large number of genes in response to stress conditions such as infection or inflammation. Cell stimuli, such as mitogens, cytokines, and viruses, cause proteolytic degradation of the I κ B subunits in the cytoplasm, leading to the subsequent translocation of NF- κ B proteins into the nucleus and the activation of various genes (25, 27).

Interestingly, the addition of PKC inhibitor prior to infection with PRRSV also significantly reduced IL-15 production in PAMs, suggesting that PKC was also required for PRRSV-induced IL-15 production. Since either PKC inhibitor or NF- κ B inhibitor alone could completely abrogate IL-15 production induced by PRRSV, it is believed that both signal pathways are essential for IL-15 upregulation, and there might exist cross talk between these two pathways. Indeed, previous reports showed that PKC could regulate NF- κ B activation, leading to the upregulation of IL-15 gene expression in RSV-infected cells (2, 19). PKC required for NF- κ B activation was also observed in airway epithelial cells for IL-8 production (43). Further studies are needed to determine whether NF- κ B activation by PRRSV infection to produce IL-15 requires PKC activation, and if so, then what the underlying mechanism is.

The IRF-E motif was demonstrated to be an essential element of the virus-inducible region of the mouse IL-15 promoter, since deletion of the IRF-E motif dramatically reduced mouse IL-15 promoter activity in mouse cell line L929 infected by Newcastle disease virus (NDV) (8). However, deletion of the IRF-E binding site only slightly reduced promoter activity induced by PRRSV and PRRSV N protein, suggesting that the IRF-E motif may be not an important element in the induction of IL-15 by PRRSV. One possible explanation is the difference that existed in the promoters. Even though both porcine IL-15 promoter and mouse IL-15 promoter have IRF-E and NF- κ B elements, IRF-E and NF- κ B elements are separated by 1 nucleotide in porcine IL-15 promoter and by 13 nucleotides in mouse IL-15 promoter (8). Another possible reason is that PRRSV infection cannot efficiently induce type I interferon production (30). The IRF-E motif is recognized by different members of the IRF family, including IRF-1, IRF-2, IRF-3, and IRF-7, which can be induced by virus infection and IFN- α/β (8).

It has been previously shown that the PRRSV N protein could activate the NF- κ B signaling pathway (36), and our results also confirmed this. We found that the PRRSV N protein can activate both the full-length IL-15 promoter (–1023/77) and the truncated promoter (–372/77). Deletion of the NF- κ B binding site in both promoters completely abrogated their luciferase activity, confirming that NF- κ B is essential for IL-15 induction. However, since the luciferase activity of full-length IL-15 promoter was low compared to that of the truncated (–372/77) promoter, we suspect that other regulatory transcription elements may exist at the region of –1023/–372. Nevertheless, based on our present data, we propose that the IL-15 upregulation was through the activation of NF- κ B signaling pathway by PRRSV N protein.

PRRSV N protein is a 15-kDa basic protein which has two nuclear localization signal (NLS) sequences at amino acids 10 to 13 (NLS-1) and 41 to 47 (NLS-2), one N-N interaction domain at amino acids 30 to 37, and one nucleolar localization signal (NoLS)

sequence at amino acids 41 to 72 (46, 47). Using serial deletion mutants of PRRSV N protein, we showed that the middle region of N protein including the N-N interaction domain, the NLS-2 domain, and the nucleolar localization signal were essential for inducing pIL-15 promoter activity. However, indirect immunofluorescence staining results showed that the location of PRRSV N protein did not affect the NF- κ B pathway activation, suggesting that PRRSV N protein may activate NF- κ B indirectly.

The fact that IL-15 expression is elevated in PRRSV-infected alveolar macrophages suggests a potential role for IL-15 in the pathogenesis of PRRSV infection. IL-15 has been proposed as an immediate response gene which becomes activated upon viral infection and serves as a signal for the recruitment of immunocytes such as NK cells and CD8⁺ T cells (3, 38, 61). Verbist et al. (56) reported that IL-15 regulated the migration of effector CD8⁺ T cells to the lung airways following influenza infection. In influenza A virus-infected IL-15 knockout (KO) mice, there were significantly fewer antigen-specific CD44⁺CD8⁺ T cells in the lungs. However, the survival rate of these mice was reduced by adoptive transfer of the CD8⁺ T cells, indicating that IL-15-dependent CD8⁺ T cells are at least partially responsible for the pathogenesis of acute pneumonia caused by influenza A virus (42). Interestingly, Samsom et al. (49) reported that during a PRRSV infection in pigs, a strong influx of NK cells and cytotoxic T lymphocytes occurred in the lungs. Consistent with this observation, high levels of IFN- γ had been detected in the lungs of PRRSV-infected pigs (9, 52, 53). Even though there are few data on IL-15 expression in the lungs of PRRSV-infected pigs, it is reasonable to speculate that IL-15 may be responsible for the influx of NK cells and cytotoxic T lymphocytes occurring in the lungs, as PRRSV primarily infects porcine alveolar macrophages of the pigs. Thus, increased numbers of T lymphocytes and NK cells in the lungs of PRRSV-infected pigs could play a role in the severe respiratory distress of these infected pigs. However, this needs to be investigated in future studies.

In conclusion, our results demonstrate that PRRSV infection could induce IL-15 production in macrophages/dendritic cells. Our data further show that upregulation of IL-15 by PRRSV requires PKC and NF- κ B pathways. In addition, PRRSV N protein is proved to have the ability to activate IL-15 production through NF- κ B activation in CRL2843 cells. The NF- κ B motif is found in the porcine IL-15 promoter region and is essential for IL-15 production induced by PRRSV and PRRSV N protein. Further research on this aspect will provide new insights into the molecular mechanism controlling the IL-15 production induced by PRRSV infection, which, in turn, should contribute substantially to a better understanding of PRRSV pathogenesis.

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