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Transmissible gastroenteritis virus does not suppress IFN-β induction but is sensitive to IFN in IPEC-J2 cells

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ABSTRACT

Coronaviruses tend to efficiently evade innate immune sensing. *Alpha-coronaviruses* interfere with the type I interferon (IFN) response in various ways, ensuring the limited activation of IFN responses. Transmissible gastroenteritis virus (TGEV), an *Alphacoronavirus* genera virus, is an important pathogen that mainly infects piglet, but little is known about the activation of the host immune response. We show that TGEV induces a delayed activation of the IFN response in intestinal epithelial cells. Briefly, IFN-β expression induced by TGEV infection is delayed with respect to that induced by poly(I:C) transfection. In addition, some of the IFN-stimulated genes (ISGs) were up-regulated in the early infection stage without obvious expression of IFN-β. Moreover, we show that activation of IFN responses induced by poly(I:C) could inhibit viral replication in the early infection stage, but failed in the late infection stage in IPEC-J2 cells. Finally, the activation of IFN responses induced by TGEV infection cannot inhibit viral replication. Taken together, this study provides a preliminary analysis of an interaction between TGEV and IFN-β responses of intestinal epithelial cells.

1. Introduction

Transmissible gastroenteritis virus (TGEV) is a kind of porcine contagious enteropathogenic coronavirus. Like other coronaviruses, it is a large, enveloped virus possessing a single-stranded, positive-sense RNA genome of approximately 28.5 kb, belonging to the genus *Alphacoronavirus* within the family *Coronaviridae* of the order *Nidovirales*. It is a major pathogen that replicates in the cytoplasm of villous epithelial cells in the small intestine, leading to watery diarrhea, dehydration and vomiting in piglets less than two weeks old (Enjuanes et al., 1995). The intestinal epithelial cells are the first line of defense against TGEV infection, therefore, intestinal epithelial interferon response is critical for resistance to TGEV infection.

Type I interferon (IFN) plays a critical role both in the innate and in the adaptive immune responses against viral infections. Double-stranded (ds) RNA that is produced by the replication of RNA virus genomes could be recognized by Toll-like receptors (TLRs) in endosomes or RIG-I-like receptors (RLRs) in the cytoplasm (Arpaia and Barton, 2011; Loo and Gale, 2011), which lead to the synthesis and secretion of IFNs. Consequently, the secretion of IFNs induces a wide array of IFN-stimulated genes (ISGs) to exert antiviral effects (Wong and Chen, 2016). However, in the competition between virus and host cells, many viruses have evolved the ability to evade or suppress the antiviral immunity (Katze et al., 2002).

It is important to avoid induction or to counteract the IFN responses for a virus replication and infectious progeny production in cells. Coronavirus has evolved a lot of strategies to resist the host innate immune responses, such as encoded proteins to affect type I IFN and proinflammatory cytokines production (Narayanan et al., 2015; Totura and Baric, 2012). Moreover, a common feature of *alpha-* and *beta-coronaviruses* is their ability to shield dsRNA and other viral components from recognition by host PRRs by intracellular membrane rearrangements. It has been reported that coronavirus nsp16 can modify the 5′ cap of coronavirus mRNAs by its 2′-*O*-methylase activity, which makes coronavirus mRNAs indistinguishable from host mRNAs (Zust et al., 2011).

IPEC-J2 is a non-transformed columnar epithelial cell line that was isolated from neonatal piglet mid-jejunum in 1989 by Helen Berschneider (Vergauwen, 2015). This primary cell line has been used increasingly to characterize epithelial cell interactions with enteric bacteria and viruses (Brosnahan and Brown, 2012). While the viral pathogenesis and vaccine development are being aggressively pursued, it is unclear why the viral replication is very fast, leading to short incubation period and swift death. Some

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studies have demonstrated that TGEV infected swine testicular (ST) cells could induce abundant IFN-β expression (Becares et al., 2016; Cruz et al., 2013b). However, the model of the IFN response in the intestinal epithelial cells infected with TGEV is still unknown.

In this study, we evaluated the effects of IFN-β and several ISGs on IPEC-J2 cells infected with TGEV. Our data revealed that TGEV did not block RIG-I-like receptors signaling pathway to activate IFN-β expression in IPEC-J2 cells. In addition, TGEV infection induced IFN-β expression just in the peak of viral RNA replication but did not induce efficient IFN-β expression in the early infection stage. IFN-β induced by poly(I:C) decreased the replication of TGEV in the early infection stage but failed in the late infection stage.

2. Materials and methods

2.1. Cells, plasmids and reagents

IPEC-J2 cell line (Guangzhou Jennio Biotech Co, Ltd., China) used in this study was cultured in Dulbecco's Modified Eagle's Medium nutrient (DMEM from Life Technologies, Shanghai, China) supplemented with 10% fetal bovine serum (FBS, Life Technologies), 16 mM HEPES (Life Technologies), and 100 μg/ml penicillin-streptomycin (Life Technologies), and incubated in an atmosphere of 5% CO^2 at 37 °C. Cells were routinely seeded at a density of 2×10^5/mL in plastic tissue culture flasks (25 cm^2 flasks, Corning, Shanghai, China) and passaged every 3–4 days for a maximum of 25 passages. In our experiments, IPEC-J2 cells were grown on 24-,6- well or 60 mm plastic tissue culture plates (Corning) at a density of 3×10^5/well, 1.5×10^6/well or 7.5×10^6/well, respectively. Poly(I:C) (HMW)/LyoVec (InvivoGen). Anti-TGEV nucleocapsid protein (N) antibodies (Preservation of our laboratory), anti-actin antibodies (Multisciences, Hangzhou, China) and HRP-conjugated secondary antibodies (Multisciences). pISRE-TA-Luc (Beyotime Technologies). pIFNβ-Luc was conducted by traditional method inserting a 348 bp promoter sequence of IFN-β into pGL6-TA-Luc plasmid. plentiCRISPR V2 used for IFNAR1/2 knockout was conducted following the method described in Feng Zhang Lab.

2.2. TGEV propagations and infections

TGEV (SHXB strain) was provided by Jiangsu Provincial Academy of Environmental Science (JAAS), and propagated in ST cells (Mcclurkin, 1965). IPEC-J2 cells were inoculated with TGEV at a multiplicity of infection (MOI) of 5 or 0.01 for 1 h at 37 °C. The inoculum and unattached virus were removed and fresh growth medium was added. Infected cells were analyzed after the required incubation period.

2.3. Plaque assay

Confluent monolayers of ST cells grown in 6-well tissue culture plates were infected with 250 μL of serial tenfold dilutions of the virus suspension. After incubation for 1 h at 37 °C, cells were overlaid with 0.7% Sea-Plague agarose in DMEM containing 2% FBS and incubated at 37 °C. At 3 days post-infection, plaques were visualized by staining with Crystal Violet in 0.8% agar.

2.4. Quantitative RT-PCR

For quantitative reverse transcription-polymerase chain reaction (RT-PCR), total cellular RNA was extracted with TRIZOL (Life Technologies) and RNA was reverse-transcribed (TaKaRa, Dalian, China). RT-PCR was performed using the Real-Time PCR system (ABI 7500, Life Technologies, USA). Gene expression was calculated with the comparative Ct method and normalized to the endogenous levels of GAPDH. Primer sequences used for RT-PCR are listed in Table 1.

Table 1
Oligonucleotides used for quantitative RT-PCR analysis or plasmid conduct.

Name	*Primer*[a]	*Sequence (5′-3′)*
IFN-β	F	TGCATCCTCCAAATCGCTCT
	R	ATTGAGGAGTCCCAGGCAAC
gRNA	F	TTCTTTTGACAAAACATACGGTGAA
	R	CTAGGCAACTGGTTTGTAACATCTTT
RIG-I	F	TCAGAAAGAGTGTGCGGTGT
	R	TAGGGTTCTCGTTGCTGGGA
IFIT1	F	ACCAGACAGGGCTTTGCTAC
	R	CTTCTGCTTTGCTGTGGTCG
IFIT44	F	AAATGCGGTGTCTCACAGGT
	R	AAATGCGGTGTCTCACAGGT
ISG15	F	TCTAGGAGCTTTTGCCCCAC
	R	ATGCCATCATGCAGTCCCTC
GAPDH	F	CCTCAATTCAGCTGGTTCGT
	R	GGGTTGAAAGACCACCAAGA
IFNβ promoter	F	CTATCGATAGGTACCCTTGGCTTATGGTGGTT
IFNβ promoter	R	CCGGATGCCAAGCTTGCTCCACTACTCAAGTG
IFNAR1	F	CACCGATAGAGACCACGACATTTTC
IFNAR1	R	CGAAAATGTCGTGGTCTCTATCAAA
IFNAR2	F	CACCGTGCCAACTCACTACACATTA
IFNAR2	R	CTAATGTGTAGTGAGTTGGCACAAA

[a] F, forward primer; R, reverse primer.

2.5. Western blot analysis

Cells were lysed in RIPA buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% NP-40) containing a protease inhibitor cocktail (Yhermo Science). The protein concentration was determined. Equal amounts of protein were separated by SDS-PAGE and electrophoretically transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Shanghai, China). After blocking with 5% nonfat milk in Tris-buffered saline containing 0.1% Tween 20, the membrane was incubated with specific primary antibodies (1:1000), followed by incubation with appropriate horseradish peroxidase-conjugated secondary antibodies. Signals were detected using SuperSignal WestPico kit (Thermo Scientific) and subjected to Image Reader LAS-4000 imaging system (FUJIFLIM, Japan).

2.6. Luciferase assays

Cells were plated in 24-well plates overnight and cotransfected with 0.25 μg of the luciferase reporter plasmids (IFN-β-Luc, ISRE-Luc, respectively), and the Renilla luciferase construct pRL-TK (Promega Corp.), as an internal control (0.01 μg) with Lipofectamine 2000 reagent (Invitrogen Corp.) according to the manufacturer's instructions. After transfection 24 h, the cells were then infected with TGEV or transfected with poly(I:C) for indicated times. The cell lysates were harvested and luciferase activity was analyzed using a dual-luciferase assay system and a luminometer (Turner BioSystems, Inc. Sunnyvale, CA, USA) according to the manufacturer's instructions. Data represent relative firefly luciferase activity normalized to renilla luciferase activity.

2.7. Statistics

Data was presented as means ± SEM. Statistical analysis was performed using Statistical Program for Social Sciences (SPSS) 16.0. Significance was determined by Analysis of Variance (ANOVA). A P value less than 0.05 was considered to be significant, and less than 0.01 was considered to be highly significant.

3. Results

3.1. TGEV infection induced IFN-β expression in IPEC-J2 cells

In order to determine whether type I IFN is inhibited by TGEV infection in IPEC-J2 cells, we examined the mRNA expression of IFN-β. As shown in Fig. 1A and B, after infection with TGEV at MOI of 5, the mRNA of IFN-β climbed at 12 h and culminated at 24 h to 48 h post infection while infection with TGEV at MOI of 0.01, the mRNA of IFN-β climbed at 12 h and culminated at 36 h to 48 h post infection. After TGEV infected at MOI of 5 for 12 h, INF-β level was around 100 relative units, while it was about 20 relative units when TGEV infected at MOI of 0.01. Morevoer, we measured the luciferase activity of IFN-β promoter after TGEV infection at MOI of 5 or 0.01 (Fig. 1C and D). The results were similar to the mRNA expression of IFN-β. Furthermore, IFN-β promoter activity was significantly higher after poly(I:C) transfection for 6 h in IPEC-J2 cells than that after TGEV infection at MOI of 5 (Fig. 1E). According to the results of cell viability test (Fig. S1A) and the induction of IFN-β expression (Fig. S1B), the treatment of 1000 ng/ml poly(I:C) (HMW)/LyoVec, a synthetic dsRNA polymer sensed by RIG-I/MDA-5, was set as IFN-β expression positive control. Despite of the dose of infection, the peak of IFN-β mRNA was similar to that treated with 1000 ng/ml Poly(I:C) (HMW)/LyoVec after 24 h. These data indicated that the type I IFN production was not obviously inhibited by TGEV in IPEC-J2 cells. The expression of IFN-β induced by TGEV infection was slower than that induced by poly(I:C) transfection.

3.2. The similar trend of TGEV replication and IFN-β mRNA expression

The replication of TGEV in ST cells has been fully studied and characterized. To determine if TGEV infection has the same characteristic in intestinal epithelial cells, IPEC-J2 cells were infected with TGEV at MOI = 5 or 0.01. As shown in Fig. 2A and B, in single infected cycle model (MOI = 5), the virus gRNA climbed at 6 h and culminated at 24 h to 36 h post infection. In multiple infected cycle model (MOI = 0.01), the peak of viral mRNA was similar with the single infected model. However, the virus titer in a single cycle (Fig. 2C), could reach 10^6 TCID50/ml at 12 h, peaked at 36 h, and then decreased. In multiple cycle (Fig. 2D), the titer reached 2×10^6 TCID50/ml at 24 h, peaked at 36 h and then decreased gradually. These data indicated that viral replication was very fast before IFN-β expression and the trend of viral gRNA expression was similar with IFN-β mRNA expression.

3.3. TGEV infection induced ISGs expression

Due to rapid replication and the similar gRNA trends of virus and IFN-β, it seems that IFN-β failed to inhibit TGEV infection. To determine if IFN-β exert antiviral activity in TGEV infection, we examined the expression of some Interferon-stimulated genes (ISGs). The mRNA of RIG-I (Fig. 3A), IFIT1 (Fig. 3B), IFIT44 (Fig. 3C) and ISG15 (Fig. 3D) were all up-regulated after IFN-β high expression and higher than those treated with poly(I:C) (HMW)/LyoVec, which indicated the type I IFN signal pathways were not blocked by TGEV infection. Interestingly, the mRNA of RIG-I, IFIT1 and IFIT44 were transiently up-regulated at 1 h and 6 h post TGEV infection while in the later infection stage (after 12 h at MOI = 5), the mRNAs of ISGs were limited and insufficient in the early infection stage. It might be induced through peroxisomal MAVS pathway, which is required for rapid but transient induction of antiviral ISGs independent of secreted IFNs (Dixit et al., 2010). These results suggested that the innate antiviral immune responses could be induced by TGEV infection. The activation of IFN responses need abundant dsRNA.

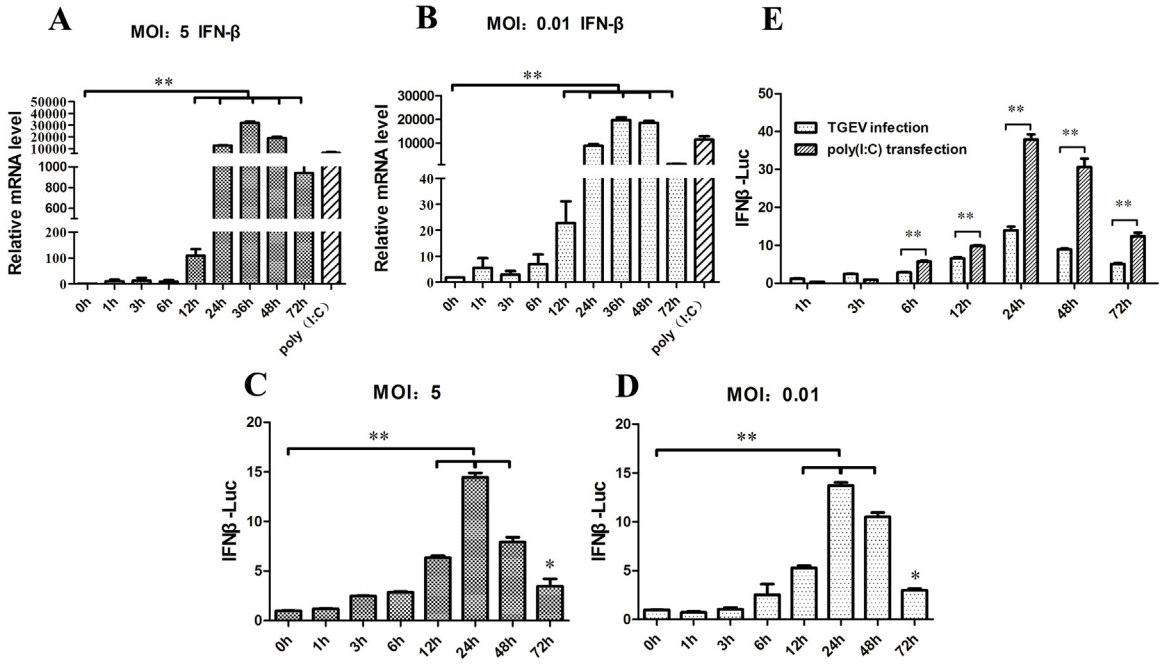

Fig. 1. TGEV infection induced IFN-β expression in IPEC-J2 cells.
IPEC-J2 cells were seeded in 24-well plates, then these cells were infected with TGEV at MOI of 5 (A) or 0.01 (B) respectively, transfection with 1000 ng/ml poly(I:C) for 24 h was a positive control. Total RNA was isolated at indicated times after infected TGEV. Real-time RT-PCR was employed to detect relative RNA expression of IFN-β. IPEC-J2 cells were seeded in 24-well plates, then these cells were co-transfected with the IFN-β promoter luciferase reporter plasmid and pRL-TK plasmid for 24 h. Cells were then infected with TGEV (MOI = 5 (C), MOI = 0.01 (D)) and harvested at indicated times for dual-luciferase assay. (E) Cells seeded in 24-well plate were co-transfected with the IFN-β promoter luciferase reporter plasmid and pRL-TK plasmid for 24 h, then these cells were infected with TGEV at MOI of 5 or transfected with poly(I:C). Cells were harvested and luciferase activity was analyzed using a dual-luciferase assay. Data are the means ± standard deviations of three independent experiments. One-way analysis of variance; *, $P < 0.05$; **, $P < 0.01$.

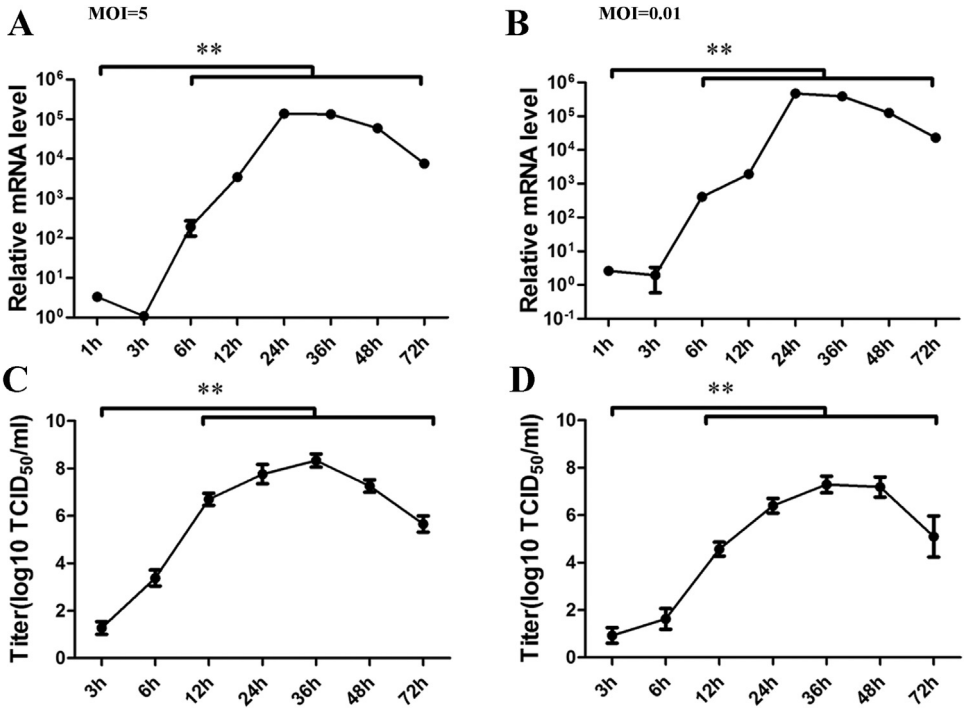

Fig. 2. The similar trend of TGEV replication and IFN-β mRNA expression.
(A and B) IPEC-J2 cells were seeded in 6-well plates, and infected by TGEV at MOI of 5 or 0.01. Total RNA was isolated after being infected at indicated times. TGEV gRNA was determined by RT-PCR. (C and D) The cell culture supernatants were harvested post-infection at indicated times and assayed for the production of infectious virus by TCID50 assay on ST cells. The TCID50 was calculated using the Reed–Muench formula. Each data point represents the average titer derived from two independent TCID50 assays. Error bars represent standard errors. One-way analysis of variance; *, $P < 0.05$; **, $P < 0.01$.

3.4. Pre-treatment of poly(I:C) (HMW)/LyoVec delayed TGEV replication

In present study, TGEV seemed not sensitive to the IFN produced, though, it has been known for more than 20 years that TGEV is sensitive to type I IFN (Weingartl and Derbyshire, 1991). In order to confirm whether IFN-β inhibit TGEV replication in intestinal epithelia cells, the IPEC-J2 cells were pre-treated with different concentrations of poly(I:C) (HMW)/LyoVec 24 h then infected with TGEV at MOI of 5 for 24 h. As shown in Fig. 4A, the replication of TGEV gRNA was significantly suppressed by poly(I:C) (HMW)/LyoVec transfected above 5 ng/ml. In order to further analyse whether activating RIG-I-like receptors (RLRs) signaling could suppress TGEV infection or not, we detected the viral gRNA, titers, and nucleocapsid (N) protein post TGEV infection at 6 h, 12 h, 24 h, 48 h, and 72 h. As shown in Fig. 4B, the viral gRNA was suppressed at 24 and 48 hpi after 500 ng/ml poly(I:C) (HMW)/LyoVec transfection. However, the trend of titers and N protein was not similar with the viral gRNA trend (Fig. 4C–E). The titers and N protein expression were suppressed by poly(I:C) (HMW)/LyoVec transfection in the early infection stage. These data indicated that the activation of IFN-I signaling pathway might inhibit TGEV infection through suppressing protein translation and virus assembly in the early stage but fail to inhibit TGEV infection on the late infection stage.

3.5. The antiviral activity of IFN-I induced by TGEV infection could not inhibit viral replication

We have mentioned above that TGEV seemed not sensitive to the IFN produced, though pre-treatment poly(I:C) could delay the viral replication. To specifically demonstrate the antiviral activity of IFN-β during TGEV infection, we tried to establish IFN-α/β receptor (IFNAR) knockout (KO) cell lines by gRNA-guided CRISPR/Cas9 knockout strategy. Through single cell pick-up and expansion, we got several IFNAR KO ST cell lines. We also conducted the relevant experiment in IPEC-J2 cells, but did not get an IFNAR KO cell line as this cell cannot grow by single cell. To determine the efficiency of IFNAR knockout, we measured the mRNA level of IFN-β and two ISGs after transfection with poly(I:C) in these IFNAR KO ST cell lines. As shown in Fig. 5, the level of IFN-β (A) was significantly suppressed and DDX58 (B), ISG15 (C) were all completely inhibited in IFNAR2-6, 7, 9 ST cell lines. Therefore, we chose IFNAR2-6 as a successful IFNAR KO ST cell line, while the IFNAR1-3 as a plasmid transfection control. Moreover, we measured the activity of IFN-β promoter and ISRE (interferon-stimulated response elements) by Luc-based reporter assays. Though, it was shown that the level of IFN-β (Fig. 5D) increases slightly induced by poly(I:C), the activity of ISRE (Fig. 5E) was completely inhibited in IFNAR2-6 cells, which indicated IFN-I responses were blocked. We measured the titer of TGEV after infection for 12 h in IFNAR KO ST cells transfected with or without poly(I:C). As shown in Fig. 5F, the replication of TGEV in IFNAR2-6 cells was similar to that in IFNAR1-3 cells or ctrl cells and it has no difference in IFNAR2-6 cells transfected with or without poly(I:C), while poly(I:C) transfection can inhibit the replication of TGEV completely in ST ctrl cells or IFNAR1-3 cells. Therefore, we can get the conclusion directly that the antiviral activity of IFN-I during TGEV infection is limited.

4. Discussion

In this study, we performed a further analysis of the replication of TGEV in intestinal epithelial cells and studied the mechanisms by which the type I IFN response interferes with TGEV infection. We showed that TGEV infection lead to a considerable activation of the type I IFN response in IPEC-J2 cells. However, the level of IFN-β was still very low even after 12 h TGEV infection (Fig. 1A), while the

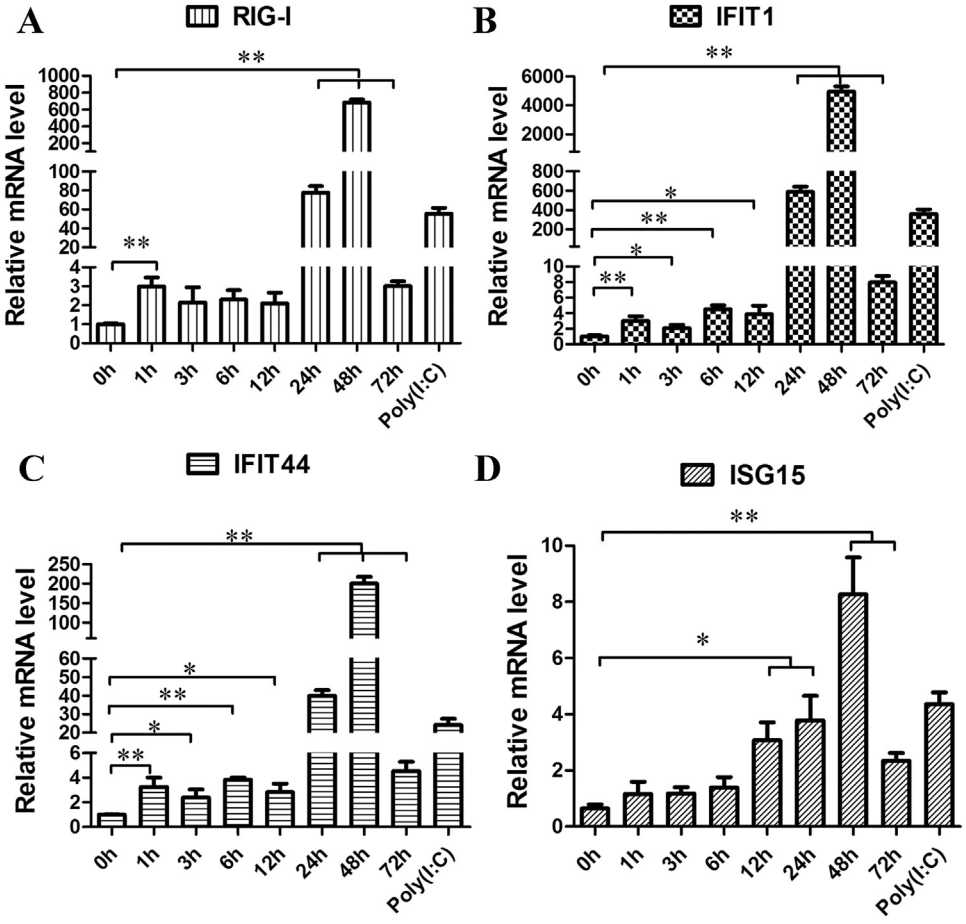

Fig. 3. TGEV infection induced ISGs expression.
IPEC-J2 cells were seeded in 24-well plates, then these cells were infected with TGEV at MOI of 5, and the treatment with 500 ng/ml poly(I:C) was a positive control. Total RNA was isolated at indicated times post TGEV infected. Real-time RT-PCR was employed to detect relative RNA expression. Fold changes of the expression RIG-I (A), IFIT1 (B), IFIT44 (C) and ISG15 (D) were shown. Data are the means ± standard deviations of three independent experiments. One-way analysis of variance; *, $P < 0.05$; **, $P < 0.01$.

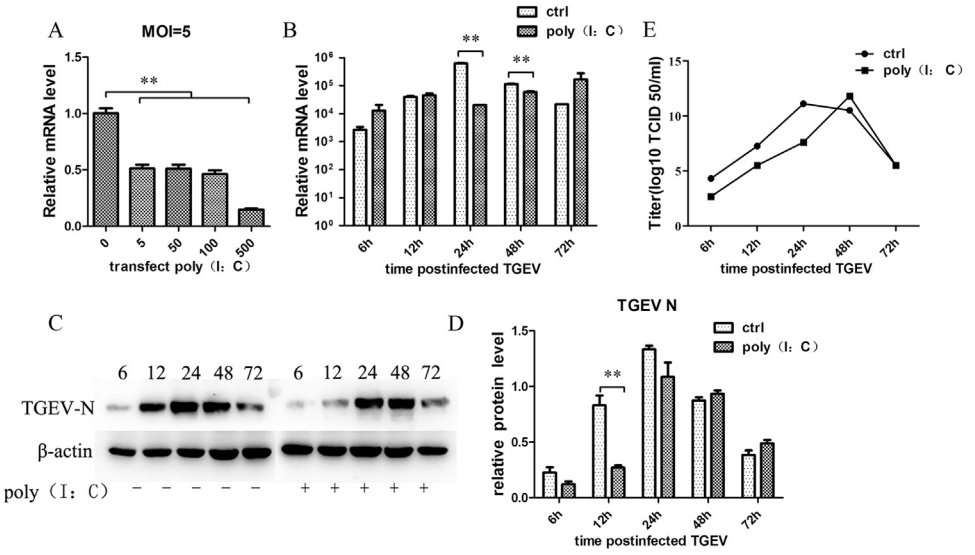

Fig. 4. Pre-treatment of poly(I:C) (HMW)/LyoVec delayed TGEV replication.
(A) After transfected with (0–500 ng/ml) poly(I:C) 24 h, IPEC-J2 cells were infected by TGEV at MOI of 5 for 24 h. The expression of TGEV gRNA was determined by RT-PCR. (B) After transfected or not transfected with poly(I:C) 24 h, IPEC-J2 cells were infected TGEV for 6–72 h. The expression of TGEV gRNA was determined by RT-PCR. (C and D) TGEV nucleocapsid (N) protein and actin expression (loading control) were analyzed by Western blot using specific antibodies. (E) The cell culture supernatants were harvested post-infection at indicated times and assayed for the production of infectious virus by TCID50 assay on ST cells. The data represent the mean ± SD of three independent experiments. One-way ANOVA; *, $P < 0.05$; **, $P < 0.01$.

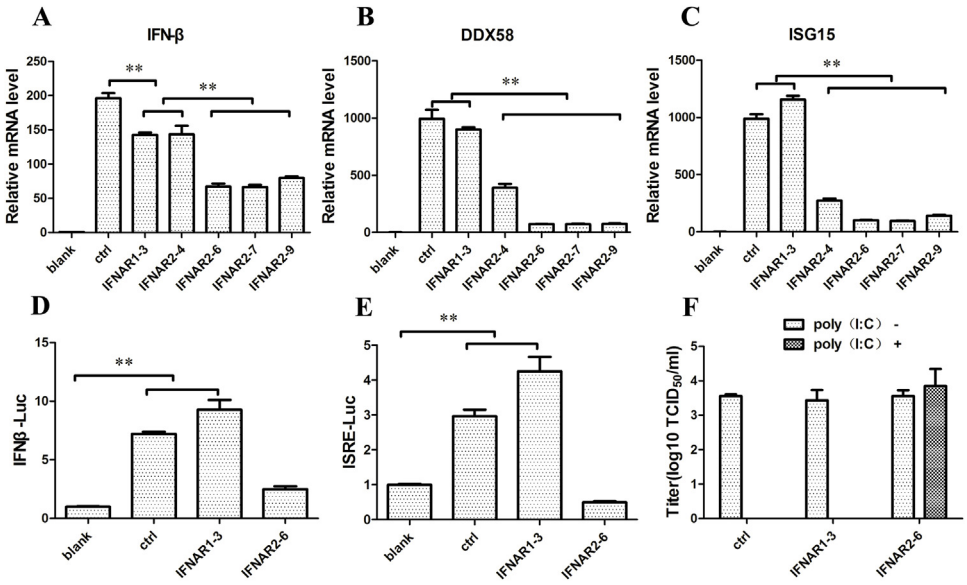

Fig. 5. The antiviral activity of IFN-I induced by TGEV infection was limited to viral replication.
IFNAR KO ST cell lines were seeded in 24-well plates overnight, then cells were transfected with poly(I:C) or not (blank group) for 24 h. Total RNA was isolated. Real-time RT-PCR was employed to detect relative RNA expression. Fold changes of the expression IFN-β (A), DDX58 (B), ISG15 (C) were shown. IFNAR KO ST cell lines seeded in 24-well plate were co-transfected with pRL-TK plasmid and the IFN-β promoter luciferase reporter plasmid (D) or pISRE-TA-Luc (E) for 24 h, then these cells were transfected with poly(I:C) or not. Cells were harvested and luciferase activity was analyzed using a dual-luciferase assay. (F) IFNAR KO ST cell lines were transfected with poly(I:C) or not for 24 h, then these cells were infected with TGEV at MOI of 0.01 for 12 h. The cell culture supernatants were harvested post-infection at indicated times and assayed for the production of infectious virus by TCID50 assay on ST cells. The data represent the mean ± SD of three independent experiments. One-way ANOVA; *, $P < 0.05$; **, $P < 0.01$.

level of TGEV titer almost get to the peak at the same time (Fig. 2C). Consequently, the production of IFN-β delayed with respect to the peak of viral titer. In addition, TGEV alone could effectively promote IFN-β induction and TGEV could enhance IFN-β production induced by poly(I:C) transfection (Fig. S2), though it could not suppress the replication of TGEV in the early stage. Finally, we demonstrated that the activation of IFN responses induced by TGEV infection cannot inhibit viral replication (Fig. 5F). Taken together, this study provides the novel analysis of the interplay between TGEV and the type I IFN response in porcine intestinal epithelial cells.

Diseases caused by coronavirus is a hot research topic in recent years, such as Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS), and Porcine Epidemic Diarrhea (PED). Because of various structural proteins and non-structural proteins, there are complex interactions between coronavirus and host cells especially for the innate immune response (Kindler and Thiel, 2014; Fung and Liu, 2014). The innate immune system is the first line of defense against virus infection, especially type I interferon antiviral response. Type I interferon (IFN), often triggered by viral infection, could broadly stimulate antiviral cytokine and inflammatory cytokines expression (Katze et al., 2008). However, induction of IFN expression is often failed in coronavirus infection (Kindler and Thiel, 2014). Resent study about PEDV indicated that the IFN-β production was inhibited by blockage of the RIG-I-mediated pathway in porcine intestinal epithelial cells (Cao et al., 2015). Despite the similarity in infection and pathological characteristics between PEDV and TGEV, several studies indicated TGEV infection could induce IFN-β expression (Becares et al., 2016; Cruz et al., 2013a, 2011; Marquez-Jurado et al., 2015). Our results also confirmed that TGEV infection induces type I interferon expression in the intestinal epithelial cells. Moreover, the trend of viral gRNA expression was similar with IFN-β mRNA expression (Figs. 1 A and 2 A). The similar trend of gRNA expression and IFN-β mRNA level was also observed in ST cells infected with TGEV (Cruz et al., 2013a). However, the abundant IFN-β was produced too late to inhibit viral replication and infection of cells. This may also be the reason for the short latency and rapid development of transmissible gastroenteritis. Since the same structure of eukaryotic mRNAs, the nucleic acid of coronavirus could not be recognized by pattern recognition receptors (e.g. TLR7/8 or RIG-I/MDA5) (Zust et al., 2011). Moreover, Knoops and Kikkert et al. reported that ER-derived double membrane vesicles (DMVs) was the main sites for SARS-CoV replication. The viral dsRNA is mostly located in the inner lumen of DMVs (Snijder et al., 2006), whereas RIG-I/MDA5 cannot efficiently detect it. Not only that, coronavirus encodes several ribonucleases, such as nsp14 (Becares et al., 2016; Bouvet et al., 2012) and nsp 6 (Lundin et al., 2014) that could remove the RNA-PRRs complex and blockage of RLRs signaling. Due to these strategies, coronaviruses have been shown to induce limited expression of IFN-β. Furthermore, SARS-coronavirus ORF9b triggers the degradation of MAVS/TRAF3/TRAF6 signalosome and limits host cell IFN responses (Shi et al., 2014). In addition, nsp5, which encodes the 3C-like protease of PEDV, disrupts type I IFN signaling by cleaving NEMO (Wang et al., 2016). In agreement with current knowledge, TGEV is just less efficient than other coronaviruses, such as SARS-CoV or MERS-CoV, antagonizing the host cell innate immune response. However, it is still confusing that the expression of IFN-β was paralleled with the increase of viral RNA during the peak of viral nucleic acid replication. However, the invasion of TGEV could not induce efficient type I IFN expression in the early infection stage.

We found that in the early infection stage RIG-I (DDX58), the main PRRs identified dsRNA, appears to rise, which may be the reason for the synergistic effect in expression of IFN-β. We can observe several ISGs increase in the early infection stage (Fig. 3). It might be the reason that peroxisomal (RIG-I-like receptor (RLR) adaptor protein) MAVS induces the rapid interferon-independent expression of defense factors that provide short-term protection (Dixit et al., 2010). However, pre-treatment with poly(I:C) could decrease the expression of gRNA (Fig. 4A and B), inhibit the level of N protein after TGEV infection 12 h (Fig. 4C) and suppress the level of viral titer (Fig. 4E). It is surprising that the adequate IFN-β and ISGs transcription could not suppress viral replication in the late

infection stage (Fig. 4). We know very little about the interaction between TGEV infection and the host innate responses although it was reported that TGEV nsp14 and accessory gene 7 had the ability to modulate the innate immune response (Becares et al., 2016; Cruz et al., 2013a). Currently, we could only speculate about the cause of this effect. It appears that some structural or non-structural protein of TGEV were highly expressed in the late infection stage, which blocked type I IFN signal, resulting in loss of antiviral activity. Moreover, in the later phase of TGEV infection, massive IFN-β expression of intestinal epithelial cells could not only fail to inhibit the viral transmission but also induce immunosuppressive molecules (McNab et al., 2015) or damage the host (Sadler and Williams, 2008; Chawla-Sarkar et al., 2003), which is not conducive to the host against virus infection.

Taken together, this study provides the model of TGEV replication and induction of IFN-β in intestinal epithelial cells. Our study characterizes that TGEV delayed IFN-β expression in early infection but promoted IFN-β expression in the peak of replication. Clearly, our understanding about the antagonistic effect to the IFN signal in late infection stage is still insufficient. Therefore, further work investigating the mechanisms of blockage of the type I IFN signal in intestinal epithelia cells during TGEV infection might be of importance to control viral infection.

Conflicts of interest

There is no conflict of interest. No writing assistance was utilized in the production of this manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vetmic.2016.12.031.

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