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Original article

Participation of interferon type I during canine parvovirus infection

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Abstract

Canine parvovirus (CPV) is a single-stranded DNA virus that causes severe and fatal gastrointestinal diseases in dogs. CPV has developed several strategies to evade innate immune response mediated by type I interferons (IFN-I) to achieve a successful infection. The aim of this work was to evaluate the capability of CVP-2c to evade the IFN-I mediated response in infected cells. To establish the role of this response, the gene expression of interferon β (IFN β), IFIT1, IFIT3, MAVS, and STING were estimated in MDCK cells infected with CPV-2c. Viral replication and gene expression was evaluated by quantitative PCR, also, a treatment with IFN-I (interferon omega) was included to confirm the role of IFN-I during CPV infection. The results revealed that CPV-2c infection stimulates the expression of IFN β moderately, in these cells. Due to low IFN β induction, the IFIT1 and IFIT3 expression were also low, and therefore CPV-2c was able to replicate in these cells. However, when the cells were treated with exogenous IFN-I, the IFN β expression was higher, leading to an increased gene expression of IFIT1 and IFIT3, responsible for antiviral control. The overexpression of these proteins reduced the expression of NS1 and VP2 viral genes and hence viral replication. MAVS and STING expression on infected cells showed a mild increase compared to IFN β , suggesting that the viral infection could partially modify its expression. All results obtained in this study showed that during CPV-2c infection in MDCK cells, the IFN β expression was altered since this cytokine is one of the most critical factors for the control and inhibition of viral replication.

Key words: canine parvovirus, antiviral immunity, IFN β , viral pathogenesis

Introduction

The innate immune response against viral infection demands recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs). PRRs include Toll-like Receptors (TLRs), RIG-I-Type (RLRs), NOD-Type (NLRs), and intracellular nucleic acid receptors (Lee and Kim 2007). Some of these intracellular sensors have a helicase domain that attaches directly to viral nucleic acid, retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 receptors (MDA5) bind RNA, and Z-DNA-binding protein 1 (DAI), Interferon Gamma Inducible Protein 16 (IFI16), cyclic GMP-AMP Synthase receptors (cGAS) bind to DNA (Takeuchi et al. 2010). RIG I and MDA5 contain two caspase recruitment domains (CARD) in the N-terminal region. When these receptors bind the viral nucleic acid undergoing a conformational change, it allows them to associate with Mitochondrial Antiviral Signaling Protein (MAVS) through CARD-CARD domain interactions (Akira and Takeda 2004). MAVS and stimulator of interferon genes protein (STING) are scaffolds that recruit and activate TRAF Family Member Associated NFKB Activator (TANK) and kinase-binding protein-1 (TBK1), this last protein is essential for the phosphorylation of transcriptional factor IRF3 (interferon regulatory factor 3), involved in IFN-I expression (Zevini et al. 2017). IFN-I stimulates the expression of many Interferon Stimulated Genes (ISGs), such as Interferon Induced Protein with Tetratricopeptide Repeats proteins (IFITs) and PRRs (Liu et al. 2011). IFIT1 binds directly to viral RNAs, blocking its translation, thus has a crucial antiviral role (Kumar et al. 2014). In response to these immune pressures, many viruses developed strategies to inhibit this antiviral innate immune machinery. These viral countermeasures block components of the pathways involved in IFN-I production, thereby contributing to these agents' pathogenesis and virulence (Grekova et al. 2010).

It is unclear whether parvoviruses represent triggers or are targets of the innate antiviral machinery. A previous study with Minute Virus of Mice (MVM) showed that although murine embryonic fibroblasts (MEFs) from C57BL/6 and CD1 mice produced IFN-I during infection, this was not enough to control viral replication (Mattei et al. 2013). Experiments made with Canine Parvovirus (CPV) in other cells lines obtained similar results (Schlehofer et al. 1992). MVM and CPV are members of the Parvoviridae family, a non-enveloped single-stranded DNA virus (Pollock 1982). The CPV genome contains two large open reading frames (ORFs) that encode two nonstructural (NS1 and NS2) and two structural (VP1 and VP2) proteins, tran-

scribed by only two mRNAs (Zhou et al. 2017). The NS1 is a multifunctional protein responsible for cytotoxic activities, cellular regulation of transcription, and cell death induction (Gupta et al. 2016). Cell death induced by members of the genus Parvovirus was cell-type dependent and involved the mitochondria (Chen et al. 2011). Parvoviruses are known to interfere with mitochondrial functions, but the role of mitochondrial proteins involved with the innate immune response during infection is only partially understood (Nykyk et al. 2014)

In the present study, MDCK cells were infected with CPV-2c. The expression of IFN β , IFIT1, IFIT3, MAVS, and STING was measured to evaluate the capability of CPV-2c to evade the IFN-I dependent cellular innate immune response. To determine the importance of this immune response during CPV-2c infection, INF-I was added before infection, and the observed response was compared against cells without treatment.

Materials and Methods

Cell lines and virus strain

MDCK (Madin-Darby canine kidney cells) were grown in MEM (Gibco, USA) cell culture medium supplemented with 10% fetal bovine serum (FBS, Gibco, USA), 1 mM HEPES and 0.1% antibiotic-antimycotic solution (AAS) (Hyclone, USA), at 37°C. The Canine parvovirus type 2c strain (CPV-2c) was previously recovered from a stool sample of a case of canine haemorrhagic gastroenteritis (Amaro 2003).

Viral infection assays

MDCK cells were infected with CPV-2c inoculum for 2 hours at 37°C. After this time, the inoculum was removed, and the cells were washed with PBS. Then, 1.5 mL of MEM with 10% FBS, 1 M HEPES, and 0.1% AAS was added, and the cells were incubated at 37°C. For recovering viral DNA from infected cells, the medium was removed, and the cells were washed with PBS. Cells were lysed with 500 μ L of Total Lysis Solution (TLS: 5 M guanidine isothiocyanate, 100 mM TRIS / HCL pH 6.4, 50 mM EDTA pH 8, and 2.5% Triton 100X). The lysate was recovered, and the DNA was extracted using silica gel. DNA obtained was suspended in 20 μ L of TE (10 mM TRIS HCl pH 8.0, 2 mM EDTA) and placed at -70°C. For viral quantification through quantitative PCR (qPCR), the NS1 gene was amplified by PCR (PCR Master Mix Fermentas, USA), purified (GeneJET Gel Extraction Kit, Thermo Fisher Scientific, USA), and quantified by spectrophotometry (Spectrophotometer Beckman model DU720). This DNA was

Table 1. Primers used to evaluate viral infection and immune response*.

Primer name	Sequence	Amplification size
VP2-CPV F	5'-TGA GGC GTC TAC ACA AGG G-3'	VP2 (96pb)
VP2-CPV R	5'-GAT CAC CAT CTG CTG CTT GA-3'	
NS1-PVC F	5'-ATG TCT GGC AAC CAG TAT ACT G-3'	NS1 (130pb)
NS1-PVC R	5'-CAG CAG ACA TCC TTT CCA TT-3'	
IFN β -can F	5'-AGC AGC AGT TTG GAG TGT CA-3'	IFN β (123pb)
IFN β -can R	5'-CTT CTG GAA CTG GCG TGA-3'	
IFIT1-can F	5'-ATA GAG GCT GGC GAC TAC AG-3'	IFIT1 (123pb)
IFIT1-can R	5'-TCC TTA CAG CCA GTT TCT CC-3'	
IFIT3-can F	5'-GGT CTA CTA TCA CCT GGG CAG A-3'	IFIT3 (137 pb)
IFIT3-can R	5'-TTT AGC CGT GTC CAC CCT T-3'	
MAVS-can F	5'-TGC CTT ACA ACA AGC GAC C-3'	MAVS (194pb)
MAVS-can R	5'-GAC TAG CCA GCT CAC AGA CC-3'	
STING-can F	5'-ACT GCC GCC TCA TTG TCT AC-3'	STING (130pb)
STING-can R	5'-GCT GCC CAT AGT AAC CTC CC-3'	
GDPH-can F	5'-GGC TGA GAA CGG GAA ACT T-3'	GDPH (78 pb)
GDPH-can R	5'-CCA TTT GAT GTT GGC GG-3'	

* All primers were designed for the present study.

diluted and employed as the standard curve in a qPCR assay (Maxima SYBR Green/ROX qPCR Master Mix, Fermentas, USA) to determine the number of viral genome copies per mL (vgc/ml) by absolute quantification (GSC calculator, <http://cels.uri.edu/gsc/cndna.html>). Three different MOI (multiplicity of infection): 100 (high), 50 (medium), and 10 (low) viral genome copies by cell (vgc/cell) were used to evaluate variations in gene expression. Also, to establish the role of IFN-I during CPV-2c viral infection, MDCK cells were treated previously with 100,000 units/well of exogenous INF-I (recombinant feline interferon omega, Virbac).

Analysis gene expression

The expression of IFN β , IFIT1, IFIT3, MAVS, STING, NS1 and VP2 was quantified. RNA from infected cells was recovered using TRIZOL (Invitrogen) following the manufacturer protocol. The cDNA synthesis was carried out using a commercial kit (AMV cDNA synthesis New England Biolab) with random primers. For the quantification assays, SYBR Green (Maxima SYBR Green/ROX qPCR Master Mix, Fermentas, USA) was used. The qPCR cycling conditions for all primers were: 30 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. All expression assays performed in this study included a normalizing gene (canine glyceraldehyde 3-phosphate dehydrogenase, GDPH). The sequence of primers used are shown in Table 1.

Statistical analysis

Three independent assays were analysed by the $2^{-\Delta\Delta CT}$ method to adjust the estimation of increases in the expression of the quantified genes (Livak and Schmittgen 2001). An analysis of variance (ANOVA) was performed to establish the significant differences between the means of each infection time. The differences between NS1, VP2, IFN β , IFIT1, IFIT3, MAVS, and STING expression were established using a factorial arrangement. For genome copies, absolute quantification was done, and ANOVA analysed the mean of each time. p-value of <0.05 was considered statistically significant. All analyses were carried out by R, R-studio, and JMP Pro 14 software (JMP Statistical Software).

Results

Kinetics of viral replication of CPV-2c in MDCK cells

To describe the replication kinetics of CPV-2c, in vitro infection assays were carried out with different MOI (high, medium, and low). The results suggest that the infection's intensity varies according to the MOI used, however, the pattern in the viral replication remains. A higher number of vgc/ml was observed on day 8 in all treatments, with values of 4.12×10^7 , 2.80×10^6 , and 1.38×10^6 vgc/ml for high, medium, and low MOI, respectively (Fig. 1). On day 14, the number of vgc/ml was reduced in all treatments, showing values of 3.06×10^7 for high, 1.25×10^6 for medium, and 2.43×10^5

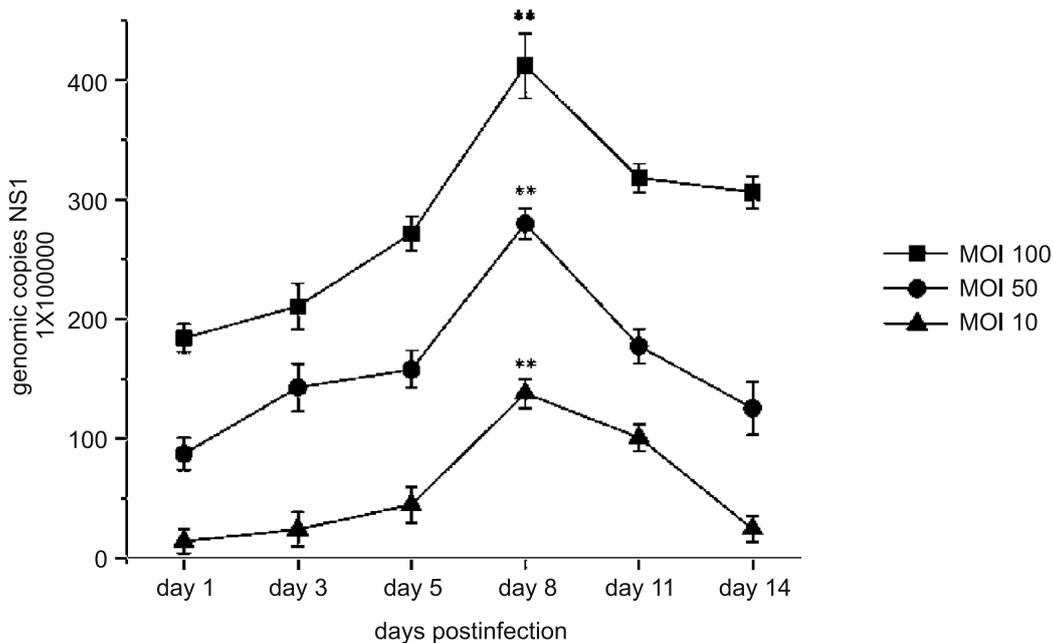


Fig. 1. CPV-2c replication kinetic curve. MDCK cells were infected with CPV-2c at different MOI. ** $p < 0.01$.

for low MOI, respectively (Fig. 1). These results indicated that the intensity of CPV-2c viral replication in MDCK cells was related to the MOI.

Analysis of CPV-2c gene expression

A quantification assay of NS1 and VP2 expression was done in MDCK cells infected with CPV-2c to confirm the previous results. For these assays, only high and low MOI doses were considered. The results showed a different expression pattern between both genes despite the MOI. In infection assays with high and low MOI, the expression of the NS1 gene reached the maximum level on day 8 post-infection (13,026- and 4.74- fold increase, respectively) and decreased on day 11 (304.32- and 1.32-fold increase, respectively) (Fig. 2A). Unlike NS1, the expression of VP2 showed a distinct pattern, this gene was overexpressed on day 8 (333.53- and 1.59-fold increase for high and low MOI, respectively) (Fig. 2A) and reached the maximum expression value on day 11 post-infection (33,096- and 3.23-fold increase to high and low MOI, respectively) (Fig. 2A). For this gene, a reduction in expression never was observed, like with NS1 gene expression.

Effect of INF-I on CPV-2c viral replication

For establishing the importance of the immune response in the control of CPV-2c, INF-I was added (100,000 U/well) before CPV-2c infection. In cells infected with high MOI and not treated with INF-I, the number of viral genome copies observed on day 3 post-infection was 2.10×10^7 vgc/ml, on day 8 was 4.12×10^7 vgc/ml and on day 11 was 3.18×10^7 vgc/ml.

A clear peak was observed in the number of genome copies of CPV-2c on day 8, as previously mentioned (Fig. 1). Cells treated with exogenous INF-I and infected with CPV (high MOI) showed different results. In these cells, the number of genomic copies decreased in all times. On day 3 post-infection, a copy number of 1.5×10^7 vgc/ml was observed. On day 8, there were only 1.30×10^7 vgc/ml and were maintained until day 11 with a titer of 1.10×10^7 vgc/ml. There was no apparent peak observed on day 8 post-infection. Practically, in these cells, the number of vgc/ml remained the same and below the ones obtained with not treated cells. These results showed that the INF-I treatment alters the CPV-2c viral replication in MDCK cells.

Expression of NS1 and VP2 genes was evaluated in MDCK cells treated and not treated with INF-I and infected with a high MOI of CPV-2c to determine if the INF-I addition modifies the expression of these viral genes. The results showed that for not treated cells, there was an increase in expression of NS1 on day 8 (13,026-fold increase) and a decline on day 11 (304.32-fold increase) (Fig. 2B). For the VP2 gene, there was an increase expression on days 8 and 11 post-infection with 333.53- and 33,096-fold increase, respectively) (Fig. 2B). For treated cells, the gene expression of VP2 showed a slight increment of 12.78- and 17.63-fold increase on days 8 and 11 post-infection (Fig. 2B). This value compared with cells treated with INF-I revealed that the treatment with this cytokine reduced the VP2 expression until 96.16% on day 8 and 99.95% on day 11 (Fig. 2B). In NS1 expression, a reduction of 99.86% was shown on day 8 and day 11 of 90.12% concerning cells without INF-I treatment (Fig. 2B). These results

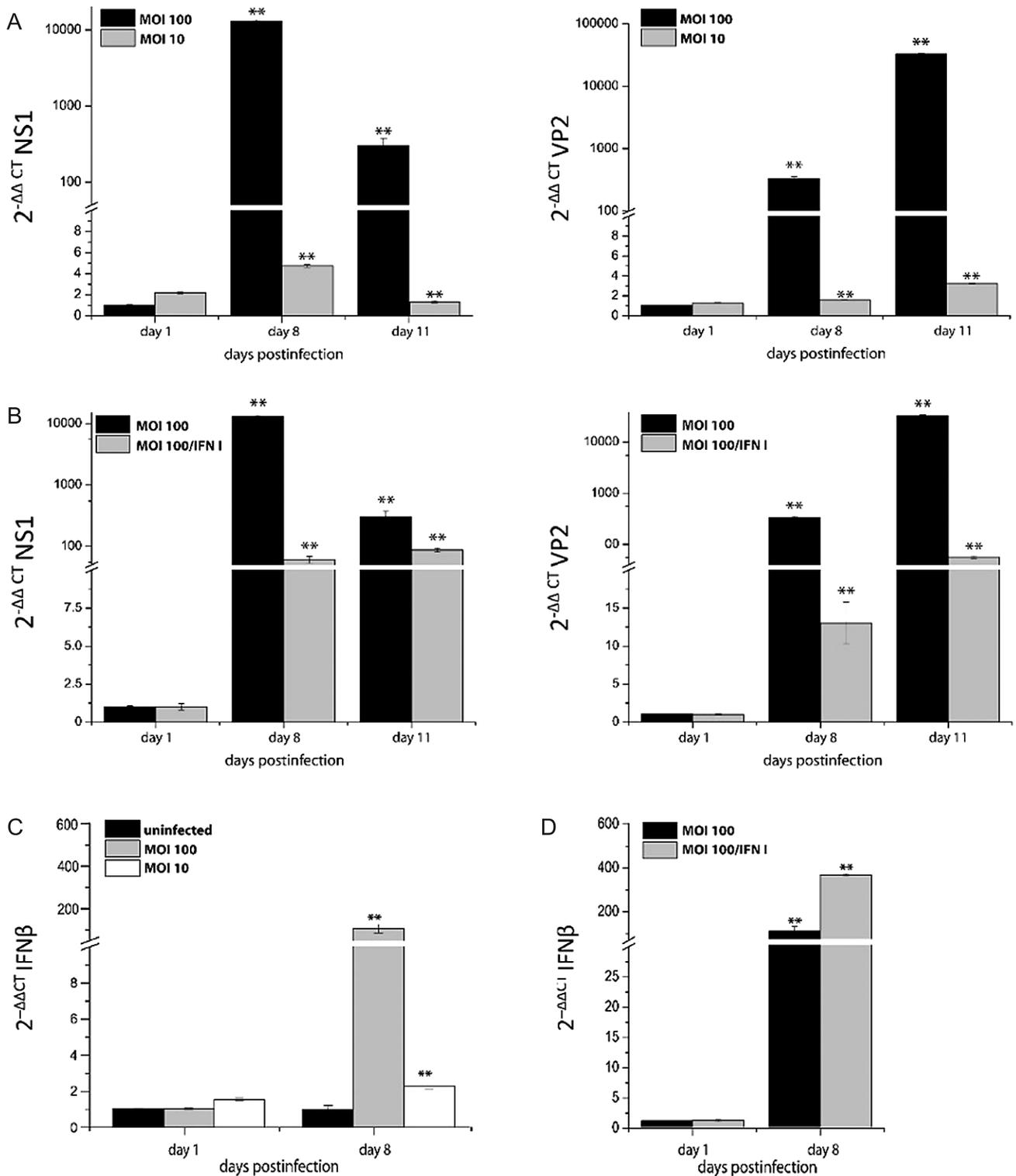


Fig. 2. Expression of viral genes and IFN β during CPV-2c infection in MDCK cells infected with two different MOI and treated with IFN-I. A) NS1 and VP2 expression on days 1, 8, and 11 post-infection in MDCK cells infected with an MOI of 100 and 10 vgc/cell, ** p < 0.01, B) NS1 and VP2 expression in treated (gray bars) and not treated with IFN-I (black bars) MDCK cells infected with CPV-2c (MOI 100 vgc/cell), C) IFN β expression in MDCK cells infected with an MOI of 100 vgc/cell (gray bars), and 10 vgc/cell (white bars), ** p < 0.001. Uninfected cells (black bars) were used as control, and D) IFN β expression in MDCK cells infected with an MOI of 100 vgc/cell and treated IFN-I (gray bars) or not treated (black bars) ** p < 0.001.

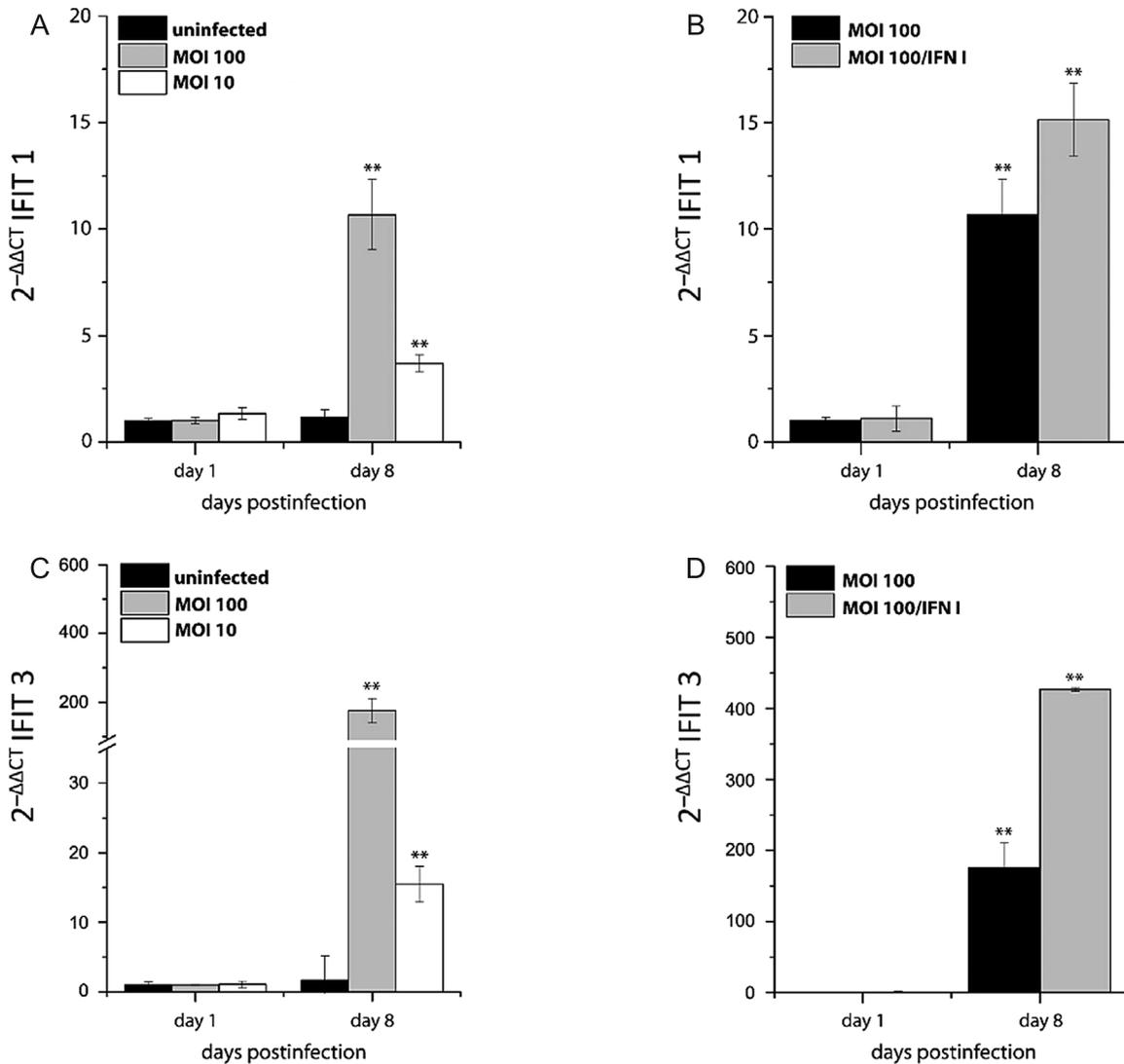


Fig. 3. Effect of IFN-I in IFIT1 and IFIT3 expression during CPV-2c infection in MDCK cells. A) IFIT1 expression in MDCK not treated cells and infected with CPV-2c at MOI of 100 vgc/cell (gray bars) and 10 vgc/cell (white bars), uninfected cells were used like control (black bars), B) IFIT1 expression in MDCK treated cells and infected with CPV-2c at MOI of 100 vgc/cell (gray bars), not treated cells were used like control (black bars), C) IFIT3 expression in MDCK not treated cells and infected with CPV-2c at MOI of 100 vgc/cell (gray bars) and 10 vgc/cell (white bars), uninfected cells were used like control (black bars), D) IFIT3 expression in MDCK treated cells and infected with CPV-2c at MOI of 100 vgc/cell (gray bars), not treated cells were used like control (black bars). ** $p < 0.001$.

showed that the treatment with IFN-I reduced the expression of both viral genes throughout the infection assays. These results accord with the low number of viral genome copies in the infection of treated cells with IFN-I.

Analysis of Interferon response in cells infected with CPV-2c

Considering that the treatment with IFN-I modifies the CPV-2c replication kinetics in MDCK cells and changes viral genes NS1 and VP2, the expression of genes involved in controlling viral infection was evaluated. First, IFN β expression assays were carried out with cells not treated and infected with high and low

MOI of CPV-2c (Fig. 2C). In cells infected with a high MOI, the expression of IFN β on day 8 was a 106.17-fold increase, and in cells infected with a low MOI, there was a 2.30-fold increase (Fig. 2C). Contrary, MDCK cells treated with exogenous IFN-I and infected with a high MOI showed a strong expression of IFN β , compared with cells infected with CPV-2c and without treatment (Fig. 2D). In these cells, the IFN β expression on day 8 had a 362.71-fold increase (Fig. 2D). The difference in IFN β expression on this day was 241.63% in cells treated with IFN-I, in comparison with cells without treatment. Assumed that expression of IFN β in cells treated with IFN-I, and infected with CPV-2c, could induce the expression of other proteins involved in the control of viral infection, expression of IFIT 1

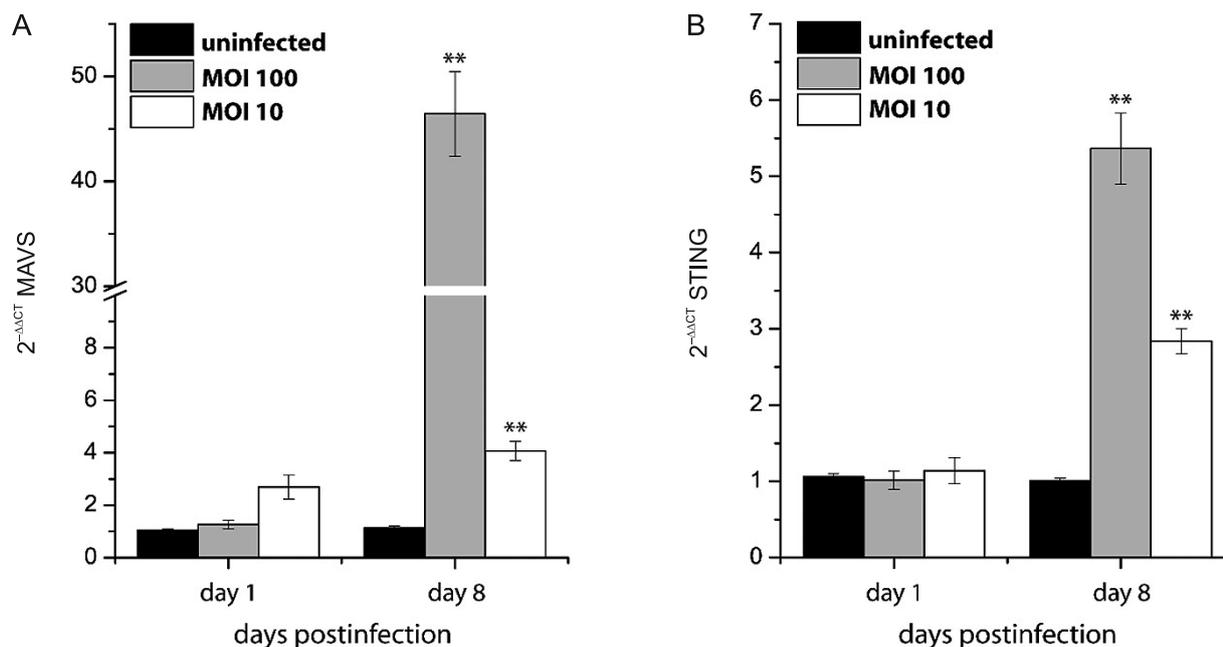


Fig. 4. Expression of MAVS and STING during CPV-2c infection in MDCK cells. A) MAVS expression in uninfected (black bars), and infected cells with CPV-2c at MOI of 100 vgc/cell (gray bars) and 10 vgc/cell (white bars); B) STING expression in uninfected (black bars), and infected cells with CPV-2c at MOI of 100 vgc/cell (gray bars) and 10 vgc/cell (white bars). ** $p < 0.001$.

and IFIT 3 was evaluated (Fig. 3). Not treated cells and infected with high MOI showed a light expression increase in IFIT 1 on day 1 (1.03-fold increase) and on day 8 post-infection (10.65-fold increase) (Fig. 3A). In cells infected with low MOI, the expression levels of IFIT 1 showed a light expression increase on days 1 and 8 (1.34- 3.69-fold increase, respectively) (Fig. 3A). Evaluation of the IFIT 3 expression in infected cells with a high MOI showed on day 1 a 1.12-fold increase and on day 8 a 175.40-fold increase (Fig. 3C). Cells infected with low MOI on day 1 had a 1.17-fold increase and on day 8 had a 15.57-fold increase for this gene (Fig. 3C). These results showed that the expression of the IFIT 3 gene is significantly higher than IFIT I in not treated cells and infected with CPV-2c. Unlike observed, in cells treated with IFN-I and infected with a high MOI, IFIT I expression showed a difference on day 8 (15.13-fold increase). In these cells, the expression of IFIT I had a 42.06% increase concerning cells without treatment (Fig. 3B). The IFIT 3 expression in IFN-I treated cells and infected with a high MOI showed a substantial increase on day 8 (425.40-fold increase). In these cells, a 142% increase compared to not treated cells treated was observed (Fig. 3D). These results demonstrated that treatment with IFN-I induces a high expression of IFIT 1 and IFIT 3 genes (Fig. 3B and 3D).

Expression of MAVS and STING was also evaluated in MDCK cells during CPV-2c infection (Fig. 4). These assays were carried out with low and high MOI, and uninfected MDCK cells were used as a control.

The results showed that in uninfected MDCK cells, expression of MAVS and STING had similar levels to GDPH (housekeeping gene), but when MDCK cells were infected with CPV-2c, an overexpression was observed (Fig. 4). Expression of MAVS in cells infected with a high MOI on day 1 had a 1.26-fold increase and on day 8, a 46.43-fold increase (Fig. 4A). For cells infected with a low MOI, the expression of MAVS on day 1 had a 2.70-fold increase, and on day 8, a 4.06-fold increase (Fig. 4A).

The STING results showed that day 1 for low and high MOI had no statistical difference between infected and non-infected cells (Fig. 4B). Only on day 8, the infected cells showed a difference in STING expression in both MOI (2.83- and 5.36-fold increase for high and low MOI, respectively) (Fig. 4B).

Discussion

Canine parvovirus (CPV) is a non-enveloped virus with a small ssDNA genome that has developed strategies to evade the host's innate immune response, facilitating its survival and proliferation. Type 1 interferons (IFN-I) exert pleiotropic biological effects during viral infections, all which contribute to virus infection control (Alcami and Koszinowski 2000). To evaluate this response during CPV-2c infection an in vitro assay were developed using MDCK cells. MDCK cells had been described as the appropriate cells for CPV isolation (Kaur et al. 2015). During this research, three different

MOI were evaluated with these cells. The results showed that during cell infection, the number of genome copies, but not the replication kinetics was dependent on the MOI, this suggests a self-control mechanism of viral replication (Fig. 1). During a virus infection cycle, many proteins are required, for CPV-2c, two non-structural proteins (NS1 and NS2) and two structural proteins (VP1 and VP2) transcribed in two long RNAs are described for this mechanism (Cotmore and Tattersall 1987). Analysis of viral transcription showed that NS1 had a higher expression on day 8, in contrast with VP2, independently of the MOI used to infect cells (Fig. 2A). These suggest that NS1 had a crucial role in viral infection in the early stages. The ectopic expression of the NS1 protein of many parvoviruses such as MVM, H-1PV, B19, and CPV2 had been reported causing cell cycle arrest, contributing to viral replication (Saxena et al. 2013). On day 11, the viral transcription analysis of VP2 showed a higher expression than NS1 (Fig. 2A), these could be due to a change in the expression level necessary for the development of new virions. The overexpression of VP2 in the late stages of infection produces an elevation of proteins required to assemble new virions that can infect surrounding cells. This phenomenon has already been described in parvovirus B19, where the early expression of non-structural proteins precedes capsid protein overexpression (Shimomura et al. 1993). Considering the observed expression patterns of NS1 and VP2 genes with high and low MOI is possible to propose that the overexpression of NS1 in the initial stages and VP2 in later stages of infection is the general pattern during CPV-2c infection in MDCK cells and suggest a control mechanism in gene expression during viral infection.

Type I interferons (IFN-I) are considered the primary antiviral response because they can activate numerous metabolic processes associated with anti-infective mechanisms (Schoggins 2014). MDCK cells were treated with IFN-I to determine the role of this cytokine during viral infection. Even when in MDCK cells, the number of CPV-2c virions necessary to carry out a successful infection was used, these were inhibited or decreased by the addition of IFN-I. The results showed that vgc/ml remained practically always below the ones observed in control cells, indicating that the addition of exogenous IFN-I modified the kinetics of viral replication. These results also confirm this cytokine's usefulness in the antiviral response and suggest that to achieve a successful infection, CPV-2c as another virus must interfere with its expression. This phenomenon has already been described in other viruses, for example, the "V" proteins of some paramyxoviruses bind to the dsRNA-sensing helicase MDA5, interfering with IFN-I

production in infected cells (Andrejeva et al. 2004). The expression of IFN β in not infected cells (control) only had basal levels, suggesting that viral infection is necessary to induce its production since the cells infected showed an overexpression. However, in MDCK cells infected with CPV-2c, the expression of IFN β seemed not enough to control the infection. The reduction in genome copies produced by the IFN-I treatment was also confirmed by changes in NS1 and VP2 expression (Fig. 2B). These results suggest that the addition of IFN-I can modify the expression of viral genes during CPV-2c infection, reducing its replication. This phenomenon had been observed with another virus, for example, exogenous administration of IFN-I reduced the replication of influenza A virus in the lower respiratory tract of *Rhesus macaques* (Matzinger et al. 2011). The innate immune response against viral infections is mediated by hundreds of interferon-regulated genes (ISGs) like the proteins with tetratricopeptide repeats (IFIT) (Weerd et al. 2007, Vladimer et al. 2014). In this study, the addition of IFN β during CPV infection could stimulate the overexpression of IFIT1 and IFIT3. These proteins' activity could explain the reduction in viral replication (Liu et al. 2011, Pichlmair et al. 2011, Kumar et al. 2014). The expression analysis for IFIT1 and IFIT3 in cells treated with IFN-I showed higher levels than in cells without treatment. Even though in cells infected with CPV-2c, an expression of IFIT1 and IFIT3 was observed, this was not enough to control the infection in MDCK cells, showing the importance of IFN through IFIT protein activities (Fig. 3). Other reports had described the antiviral activities of IFIT3 and IFIT1 during the infection of diverse viruses (Diamond and Farzan 2012), however, more research is necessary to establish these proteins' role in the control of CPV-2c.

Considering that CPV infection induces the expression of IFN β , in this study, the expression of MAVS and STING was also evaluated to describe the antiviral signaling pathway that could be involved. The results obtained suggest that MAVS expression could participate in controlling CPV-2c infection (Fig. 4). MAVS overexpression could be necessary to produce more monomers that polymerize in the mitochondrial membrane, enabling recruit transcriptional factors that stimulate the expression of IFN β (Zhong et al. 2008). In MDCK cells, overexpression of MAVS was observed only in infected cells, suggesting that stimulation by a viral infection was necessary to induce its expression. For STING expression, results showed that only a minor stimulation was observed in MDCK cells infected with CPV-2c. This result suggests that this protein is also necessary for IFN β expression, although MAVS could be the most important protein during CPV-2c infection.

The results obtained in this research demonstrated that there was a molecular modulation of the immune response during CPV-2c infection in MDCK cells. Also, IFN-I was necessary for an efficient antiviral response, associated with the expression of antiviral proteins IFIT 1 and IFIT 3, responsible for reducing viral replication. This response might be related to MAVS protein without leaving aside the participation of STING protein. Both pathways seem to converge in controlling the CPV-2c infection.

The use of IFNs for the treatment of viral infectious diseases on their antiviral activity may become an important therapeutic option. A double-blind, placebo-controlled trial with 94 dogs showed that IFN-I (recombinant feline interferon omega) therapy improved clinical signs and resulted in a 6.4-fold reduction in mortality (de Mari et al. 2003). The results obtained in our study could explain these clinical results since the treatment of cells with IFN-I reduced the viral replication and contributes to interferon expression.

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References

- Akira S, Takeda K (2004) Toll-like receptor signalling. *Nat Rev Immunol* 4: 499-511.
- Alcami A, Koszinowski UH (2000) Viral mechanisms of immune evasion. *Trends Microbiol* 8: 410-418.
- Amaro NM (2003) Determinación del parvovirus canino en heces por medio de la técnica reacción en cadena de la polimerasa, México, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México.
- Andrejeva J, Childs KS, Young DF, Carlos TS, Stock N, Goodbourn S, Randall RE (2004) The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN beta promoter. *Proc Natl Acad Sci USA* 101: 17264-17269.
- Chen AY, Kleiboeker S, Qiu J (2011) Productive Parvovirus B19 infection of primary human erythroid progenitor cells at hypoxia is regulated by STAT5A and MEK signaling but not HIF alpha. *PLoS Pathog* 7: e1002088.
- Cotmore SF, Tattersall P (1987) The autonomously replicating parvoviruses of vertebrates. *Adv Virus Res* 33: 91-174.
- de Mari K, Maynard L, Eun HM, Lebreux B (2003) Treatment of canine parvoviral enteritis with interferon-omega in a placebo-controlled field trial. *Vet Rec* 152: 105-108.
- de Weerd NA, Samarajiwa SA, Hertzog PJ (2007) Type I interferon receptors: biochemistry and biological functions. *J Biol Chem* 282: 20053-20057.
- Diamond MS, Farzan M (2012) The broad-spectrum antiviral functions of IFIT and IFITM proteins. *Nat Rev Immunol* 13: 46-57.
- Grekova S, Zawatzky R, Hörlein R, Cziepluch C, Minberg M, Davis C, Rommelaere J, Daeflfer L (2010) Activation of an antiviral response in normal but not transformed mouse cells: a new determinant of minute virus of mice oncotropism. *J Virol* 84: 516-531.
- Gupta SK, Sahoo AP, Rosh N, Gandham RK, Saxena L, Singh AK, Harish DR, Tiwari AK (2016) Canine parvovirus NS1 induced apoptosis involves mitochondria, accumulation of reactive oxygen species and activation of caspases. *Virus Res* 213: 46-61.
- Kaur G, Chandra M, Dwivedi PN, Sharma NS (2015) Isolation of canine parvovirus with a view to identify the prevalent serotype on the basis of partial sequence analysis. *Vet World* 8: 52-56.
- Kumar P, Sweeney TR, Skabkin MA, Skabkina OV, Hellen CU, Pestova TV (2014) Inhibition of translation by IFIT family members is determined by their ability to interact selectively with the 5'-terminal regions, cap0-, cap1- and 5'-ppp- mRNAs. *Nucleic Acids Res* 42: 3228-3245.
- Lee MS, Kim YJ (2007) Signaling pathways downstream of pattern recognition receptors and their cross talk. *Annu Rev Biochem* 76: 447-480.
- Liu XY, Chen W, Wei B, Shan YF, Wang C (2011) IFN-induced TPR protein IFIT3 potentiates antiviral signaling by bridging MAVS and TBK1. *J Immunol* 187: 2559-2568.
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2-Delta Delta C(T) method. *Methods* 25: 402-408.
- Mattei LM, Cotmore SF, Tattersall P, Iwasaki A (2013) Parvovirus evades interferon-dependent viral control in primary mouse embryonic fibroblast. *Virology* 442: 20-27.
- Matzinger SR, Carroll TD, Fritts L, McChesney MB, Miller CJ (2011) Exogenous IFN-alpha administration reduces influenza A virus replication in the lower respiratory tract of *Rhesus macaques*. *PLoS One* 6: e29255.
- Nykky J, Vuento M, Gilbert L (2014) Role of mitochondria in parvovirus pathology. *PLoS One* 9: e86124.
- Pichlmair A, Lassnig C, Eberle CA, Górná MW, Baumann CL, Burkard TR, Bürckstümmer T, Stefanovic A, Krieger S, Bennett KL, Rüllicke T, Weber F, Colinge J, Müller M, Superti-Furga G (2011) IFIT1 is an antiviral protein that recognizes 5'-triphosphate RNA. *Nat Immunol* 2: 624-630.
- Pollock RV (1982) Experimental canine parvovirus infection in dogs. *Cornell Vet* 72: 103-119.
- Saxena L, Kumar GR, Saxena S, Chaturvedi U, Sahoo AP, Singh LV, Santra L, Palia SK, Desai GS, Tiwari AK (2013) Apoptosis induced by NS1 gene of canine parvovirus -2 is caspase dependent and p53 independent. *Virus Res* 173: 426-430.
- Schlehofer JR, Rentrop M, Männel DN (1992) Parvoviruses are inefficient in inducing interferon-beta, tumor necrosis factor-alpha, or interleukin-6 in mammalian cells. *Med Microbiol Immunol* 181: 153-164.
- Schoggins JW (2014) Interferon-stimulated genes: roles in viral pathogenesis. *Curr Opin Virol* 6: 40-46.
- Shimomura S, Wong S, Brown KE, Komatsu N, Kajigaya S, Young NS (1993) Early and late gene expression in UT-7 cells infected with B19 parvovirus. *Virology* 194: 149-156.
- Takeuchi O, Akira S (2010) Pattern recognition receptors and inflammation. *Cell* 140: 805-820.

Vladimer GI, Góna MW, Superti-Furga G (2014) IFITs: emerging roles as key anti-viral proteins. *Front Immunol* 5: 1-6. *Front Immunol* 5: 94.

Zevini A, Olganier D, Hiscott J (2017) Crosstalk between cytoplasmic RIG-I and STING sensing pathways. *Trends Immunol* 38: 194-205.

Zhong B, Yang Y, Li S, Wang YY, Li Y, Diao F, Lei C, He X,

Zhang L, Tien P, Shu HB (2008) The adaptor protein MITA links virus-sensing receptors to IRF3 transcription factor activation. *Immunity* 29: 538-550.

Zhou P, Zeng W, Zhang X, Li S (2017) The genetic evolution of canine parvovirus - A new perspective. *PLoS One* 12: e0175035.