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Classical swine fever virus suppresses maturation and modulates functions of monocyte-derived dendritic cells without activating nuclear factor kappa B

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ABSTRACT

Classical swine fever virus (CSFV) compromises the host immune system, causing the severe disease of pigs. Dendritic cells (DCs) are the most potent inducers of immune responses. In the present study, we investigated the functional properties of porcine monocyte-derived DCs (Mo-DCs) affected by CSFV. Results showed that the expression of surface markers of DCs such as major histocompatibility complex class II (MHC-II), CD80, CD83 and CD86 were unimpaired, but an obviously increased expression of CD172a in DCs was noticed 48 h after CSFV infection. The expression profiles of cytokines were detected in cultured Mo-DCs after various treatments for 48 h by Q-RT-PCR. The findings suggested that CSFV infection significantly increased the mRNA expression of IL-10 and TNF-α, and inhibited IL-12 expression, with little effect on IFN-α and IFN-γ expression. We further demonstrated that CSFV was incapable of activating the nuclear factor kappa B (NF-κB) in infected DCs, which was characterized by an unvaried DNA binding activity of NF-κB, the lack of translocation of p65/RelA from the cytoplasm to the nucleus and the stabilization of p65/RelA expression. Furthermore, Western blot analysis indicated that the inactivation of NF-κB was due to the failure of IκBα degradation. The data demonstrated that CSFV could be replicated in DCs and CSFV infection could modulate the secretion of crucial co-stimulatory molecules and cytokines which down-regulated maturation of DCs, without activating NF-κB in DCs. Thus, the results suggested a possible mechanism for CSFV evasion of innate host defenses, providing the basis for understanding molecular pathways in CSFV pathogenesis.

1. Introduction

Classical swine fever virus (CSFV) is the highly contagious agent of severe viral disease in domestic pigs (Ruggli et al., 2009). The virus has a high affinity for cells of the immune system, and it can be replicated in myeloid cells including macrophages and dendritic cells (DCs) and interfere with cellular antiviral defense (Bauhofer et al., 2005). The invasion of CSFV to the host immune system can cause severe lymphopenia which is the hallmark of CSFV infection, resulting in immunosuppression (Jamin et al., 2008). CSFV can also penetrate the placenta to establish an infection in the developing fetus, resulting in the birth of persistently infected animals (Doceul et al., 2008). Previous studies have shown that CSFV evolved mechanisms to establish a long-term infection (Bensaude et al., 2004; Ruggli et al., 2005, 2009). Although a large number of works have been done, the immunopathological mechanisms through which CSFV interacts with the innate and adaptive immune compartments are still not completely understood.

Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs) bridging innate and adaptive immune systems with the unique capacity to initiate primary T cell responses and efficiently stimulates memory responses (Steinman and Hemmi, 2006). They are also crucial to stimulate both $CD4^+$ T helper (Th) cells and $CD8^+$ cytotoxic T lymphocytes (CTLs) (Zhou et al., 2007). Thus, their dysfunction may play a role in the disruption of host immune responses. Previous studies showed that the potential capability of DCs to modulate adaptive immunity is dependent on their maturation (Saito et al., 2008). Immature DCs located in peripheral tissues with high capability of antigen uptake are activated by a variety of factors; then they migrate into the secondary lymphoid organs, where they become mature DCs. After maturation, DCs efficiently present antigens to rare antigen-specific (Ag-specific) T cells for initiating immune responses. During DCs' maturation, a variety of DCs-specific gene expressions are induced. In particular, co-stimulatory molecules (CD80, CD83 and CD86), major histocompatibility complex class I (MHC-I) and class II (MHC- II) molecules, as well as cell adhesion molecules are up-regulated (Kruse et al., 2000; Zhou et al., 2007). Mature DCs also secrete IFN-α and IL-12, which facilitate the effective priming of type 1 cytokine-producing T cells with resistance to viral infection

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(Anthony et al., 2004). However, impaired DCs maturation and the production of immunological molecules generally result in an immunocompromised state of immune tolerance or ignorance.

In most of the cells, nuclear factor kappa B (NF-κB) is involved in a number of cellular processes, including immune regulation, inflammatory response and anti-apoptosis effect (Chen et al., 2003). NF-κB pathway is a common target of many viruses. Certain virus could make use of the activation of NF-κB as a strategy to increase viral replication and viral progeny production (Gregory et al., 2004). Whereas other reports indicated that some viruses such as human cytomegalovirus (Nachtwey and Spencer, 2008), African swine fever virus (Silk et al., 2007), foot-and-mouth disease virus (Santos et al., 2007) and torque teno virus (Zheng et al., 2007) inhibited this pathway. The suppression of NF-κB activation represents a potential strategy of an escape from the host immune system and contributes to virus pathogenesis in infected cells. CSFV infection of porcine DCs has been investigated by some groups (Carrasco et al., 2004; Bauhofer et al., 2005; Jamin et al., 2008). However, the role of CSFV in the NF-κB activation in infected-DCs has not yet been reported.

Taking into account of the role of DCs and the NF-κB pathway in the induction of innate and adaptive immune responses mentioned above, we have examined the interactions of CSFV and NF-κB in infected porcine Mo-DCs. Our results demonstrated that CSFV had an ability to suppress maturation of DCs and to modulate expression of immunological molecules in Mo-DCs, without activating NF-κB by the infection.

2. Materials and methods

2.1. Virus preparation

The virulent wild-type CSFV GXW-O7 strain, originally isolated from a lymphoid tissue sample of a pig with naturally occurring classical swine fever (CSF), was propagated on porcine kidney 15 (PK-15) cells. The titer of the virus stock was 4.06×10^5 PFU/ml as quantified by endpoint titration on PK-15 cells and immunofluorescent detection.

2.2. Generation of porcine Mo-DCs

Porcine Mo-DCs were performed as previously described (Carrasco et al., 2004; Eisemann et al., 2008). Briefly, peripheral blood mononuclear cells (PBMC) were isolated from healthy two-way crossbred pigs by density gradient centrifugation. Monocytes were removed from PBMC by plastic adherence. To generate DCs, the monocytes were cultured in RPMI-1640 complete medium (consisting of 10% FBS, 100 U/mL penicillin and streptomycin). Next, 25 ng/mL recombinant swine GM-CSF (rswGM-CSF) and 20 ng/mL recombinant swine IL-4 (rswIL-4) (BioSource) were added on the first day of culture. Half of the medium were replaced by fresh rswGM-CSF- and rswIL-4-supplemented medium every three days. After five days of culture, the cells with a dendritic morphology were defined as Mo-DCs.

2.3. CSFV infection in Mo-DCs

Mo-DCs were infected with GXW-O7 or heat-inactivated GXW-O7 (Hi-GXW-O7; 60 °C, 10 min) at multiplicities of infection (MOI) of 1 or mock infected (PK-15 cells culture supernatants) and cultured in RPMI-1640 complete medium supplemented with 25 ng/mL rswGM-CSF and 20 ng/mL rswIL-4 until they were harvested for further experiments. As the control group, Mo-DCs were treated with 1 μg/mL lipopolysaccharide (LPS; Sigma). Prior to the experiments, the presence of virus in the Mo-DCs was detected by transmission electron microscope or flow cytometric detection of the viral structural glycoprotein E2 with monoclonal antibody (mAb) (kindly provided by Dr. Qizu Zhao, China Institute of Veterinary Drugs Control). Due to the intracellular expression of E2, the Mo-DCs were fixed and permeabilized (Cell Permeabilization Kit; Harlan Sera-Lab) before labeling with the mAb.

2.4. Flow cytometric analysis (FCM) of Mo-DCs

Forty-eight hours after the infection (hpi), MO-DCs were stained with various mAbs: mouse anti-pig CD1-R-PE (Southernbiotech), mouse anti-porcine SLA-II-DR-FITC or mouse anti-pig CD172a-FITC (AbD Serotec) on ice in PBS containing 1% bovine serum albumin (BSA) and 0.01% sodium azide (pH 7.2). After 30 min of incubation, the cells were analyzed using a FACS Calibur Fow Cytometer (BD Bioscience). Meanwhile, the isotype-matched mouse antibody was added as an isotype control.

2.5. Quantitative real-time PCR (Q-RT-PCR)

At 48 hpi, total RNA of MO-DCs was extracted and quantified as previously described (Lee and Kleiboeker, 2005). The cDNA was synthesized from total RNA and an aliquot (1/10) of the cDNA was used as template for Q-RT-PCR which was performed in a 20 μL volume using FastStart DNA Master SYBR Green I mixture (Roche) for 3 min at 95 °C for initial denaturing, followed by 40 cycles at 95 °C for 30 s, 55 °C for 45 s, and 72 °C for 45 s in an ABI Prism 7500 sequence detection system (Applied Biosystems). Specific primers used for amplification are listed in Table 1. The amplification of β-actin was used as an internal control for normalization. The relative expression of target genes compared to β-actin was determined as Pfaffl method (Pfaffl, 2001).

2.6. Electrophoretic mobility shift assays (EMSA)

Nuclear protein extracts from Mo-DCs at 48 hpi were prepared with the nuclear extraction kit (Pierce) according to manufacturer's protocol. Protein concentration was determined using a BCA protein assay kit (Pierce) with bovine serum albumin as a standard. EMSA was performed using the DIG Gel Shift Kit (Roche) according to manufacturer's manual. A DIG-labeled double-stranded oligonucleotide containing a κB binding site (GGGACTTTCC) was used as a probe in these assays. Anti-p65 rabbit polyclonal antibodies

Table 1
Nucleotide sequences of primers for real-time PCR (forward primers are listed first).

Gene	Sequence of forward & reverse primers (5′–3′)	GenBank accession No.	Size (bp)
β-actin	CCTGACCCTCAAGTACCCCA GCTCGTTGTAGAAGGTGTGGTG	U16368.1	89
CD80	GGAAAAGTGAAGGTGTGGCC CATTGTCTGACAGGCGCAGA	AF455811.1	100
CD83	AACCTCAGCGGCACTGTGAT AGCCAACAGCAGGACAATCTC	XM_001928655.1	102
CD86	CTCTCTGGTGCTGCCTCCTT ATGACCAGCTCATCCAGGCT	AY826403.1	107
IFN-α	GGGACTTTGGATCCCCTCAT GAGCTGGAAGGTCTGCTGGA	AY687280.1	101
IFN-γ	GGTTCCTAAATGGTAGCTCTGGG GAGTTCACTGATGGCTTTGCG	AY293733.1	101
IL-10	AGCTGCATCCACTTCCCAAC CCCATCTGGTCCTTCGTTTG	EF522118.1	101
IL-12	ACAGAAGCCCTCCCTGGAAG CATTCTGTCGATGGTCACCG	NM_213993.1	103
TNF-α	GGACAGCTCCAATGGCAGAG AGGTACAGCCCATCTGTCGG	NM_214022.1	101