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Differential response of porcine immature monocyte-derived dendritic cells to virulent and inactivated transmissible gastroenteritis virus

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ABSTRACT

Exposure of piglets less than 2 weeks of age to virulent transmissible gastroenteritis virus (TGEV) gives rise to mortality as high as 100%, and adult pigs recovering from its infection often become TGEV carriers. These facts suggest an evasion of the immune system by virulent TGEV. In this study, we showed that a virulent TGEV SHXB strain could infect porcine immature monocyte-derived dendritic cells (Mo-DCs), and down-regulate cell surface markers (SLA-II-DR, CD1a and CD80/86). Moreover, SHXB-infected immature Mo-DCs showed low expression of IL-12 and IFN-γ, and also lost the ability to stimulate T cell proliferation. Finally, SHXB inhibited the activation of nuclear factor kappa B (NF-κB) in these cells. Instead, UV-inactivated SHXB (UV-SHXB) had the opposite effects in immature Mo-DCs. In conclusion, the virulent SHXB could severely impair immature Mo-DCs, which might be involved in the pathogenesis of virulent TGEV *in vivo*.

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1. Introduction

Transmissible gastroenteritis virus (TGEV), a member of *Coronaviridae*, order *Nidovirales* (Masters, 2006), causes a highly contagious enteric infection in pigs (Pritchard et al., 1999). The exposure of piglets less than 2 weeks of age to virulent TGEV gives rise to mortality as high as 100% within 5 to 7 days, and adult pigs recovering from virulent TGEV infection often become TGEV carriers (McGoldrick et al., 1999; Zhang et al., 2007). These clinical features strongly suggest evasion of the immune system by virulent TGEV.

Dendritic cells (DCs), the most powerful antigen-presenting cells, are critical players in intestinal immune defence. Immature DCs, with the powerful ability to capture antigens and the poor ability of presenting antigens, were widely distributed in the entire intestinal mucosa with prominent localisation in the sub-epithelial areas, forming an extensive network monitoring the invasion of pathogens (Haverson et al., 2000). On encountering antigens, these cells capture invading pathogen, and then migrate to T cell-rich areas of lymphoid organs, gradually maturing during migration. Mature DCs stimulate T cell activation and differentiation for initiating immune responses through the expression of major histocompatibility complex, co-stimulatory molecules and the production of cytokines, such as IFN-γ and IL-12 (Spörri and Reis e Sousa, 2005). Recently, reports have shown that infection of DCs with herpes simplex virus type 1 results in defective maturation, hampering the activation of naïve T cells (Boliar and Chambers, 2010). In addition, the measles virus impedes DC-derived IL-12 production, and fails to stimulate naïve T cell activation (Servet-Delprat et al., 2003). Thus, although DCs are important contributors to antiviral immunity, virus infection can impair the immune function of DCs, potentially leading to persistent infections or anergy of the immune system. TGEV mainly infects and replicates in the porcine enterocytes and may encounter sub-epithelial DCs. However, whether virulent TGEV can also impair the function of porcine DCs resulting in the severe damage of piglets is still not completely understood.

In most cells, nuclear factor kappa B (NF-κB) is involved in a number of cellular processes, including immune regulation, inflammatory response and anti-apoptosis effects (Rahman and McFadden, 2011). Some viruses have evolved strategies to interfere with NF-κB activation in order to evade the immune response and increase their replication and viral progeny production, for example, Epstein–Barr virus, hepatitis C virus, human cytomegalovirus and herpes simplex viruses (Blattman et al., 2014; Choi et al., 2006; Taylor and Bresnahan, 2006; Valentine et al., 2010). In addition, Maria Rescigno and colleagues found that inhibition of NF-κB activation could block the maturation of DCs through down-regulation of major histocompatibility complex and co-stimulatory molecules (Rescigno et al., 1998). However, the role of virulent TGEV in NF-κB activation in infected DCs has not yet been reported.

To date, scarce information is available concerning the interaction between porcine conventional DCs and virulent TGEV and

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UV-inactivated SHXB (UV-SHXB). In this study, the interaction of the virulent TGEV SHXB strain and UV-SHXB with porcine immature Mo-DCs was investigated. We showed that SHXB could infect the immature Mo-DCs, inhibit the maturation of immature Mo-DCs by down-regulating the expression of major histocompatibility complex and co-stimulatory molecules on them, also limit the production of IL-12, IFN-γ and IL-10, and finally suppress the activation of NF-κB in immature Mo-DCs. However, contrasting results were reported in UV-SHXB treated immature Mo-DCs.

2. Materials and methods

2.1. Virus stock

TGEV (SHXB, wild-virulent strain, verified by the animal attack virus test) was provided by the Jiangsu Academy of Agricultural Sciences (JAAS). The virus was propagated in Swine Testicle (ST) cells and purified using a sucrose gradient, as described previously (Krempl and Herrler, 2001). The viral 50% cell tissue infectious dose ($TCID_{50}$) of the purified SHXB strain was calculated using the Reed and Muench method (Haggett and Gunawardena, 1964). The purified virus was irradiated using UV light for 4 h at an optimal cross-linking value (0.120 J/cm^2). The inactivated SHXB (UV-SHXB) was then determined in ST cells by using an anti-TGEV polyclonal antiserum conjugated to fluorescein isothiocyanate (FITC) (VMRD, USA).

2.2. Generation of Mo-DCs

Porcine Mo-DCs were generated as previously reported (Facci et al., 2010). Briefly, porcine peripheral blood mononuclear cells (PBMC) were separated from the blood of six pigs which were negative for TGEV (2 months old, derived from a combination of the Yorkshire, Landrace, and Large White breeds, raised at an experimental animal breeding centre at JAAS, and the negativity of piglets for TGEV was demonstrated using the RT-PCR (data not shown)) by density centrifugation using Histopaque (1.077 g/L) (Sigma, USA). PBMCs were washed three times in RPMI 1640 medium (Gibco, USA), and resuspended in complete RPMI 1640 medium with 10% FBS (MULTIcell, Canada). PBMC were then placed in six-well plates and incubated overnight at 37 °C in 5% CO_2. The non-adherent cells were removed, leaving the adherent monocytes. The monocytes were cultured in complete RPMI 1640 medium containing 20 ng/mL of pIL-4 (BioSource, USA) and 20 ng/mL of pGM-CSF (Invitrogen, USA) at 37 °C in 5% CO_2. Cells were incubated for 5 days to allow differentiation into immature Mo-DCs; complete RPMI 1640 medium was replaced with cytokine-containing medium on day 3. Immature Mo-DCs were harvested on day 5 and resuspended in RPMI 1640 medium.

2.3. SHXB and UV-SHXB infection of immature Mo-DCs

In our preliminary experiment, we chose four concentrations ($TCID_{50}$ = 0.1, 1, 10 and 100) of TGEV SHXB strain interactions with dendritic cells, the best effect of TGEV infected immature Mo-DCs is the concentration of $TCID_{50}$ = 10. Thus, the immature Mo-DCs were inoculated with TGEV SHXB strain and UV-SHXB at a concentration of 10$TCID_{50}$ cell^{-1}. SHXB was adsorbed for 1 h at 37 °C. In order to eliminate the non-absorbed virus, cells were washed five times at 200 × g for 5 min at 37 °C, and resuspended in fresh RPMI 1640 medium containing pIL-4 and pGM-CSF. In addition, immature Mo-DCs were treated with lipopolysaccharide (LPS, 1 μg/mL, Sigma) for positive control, and the mock immature Mo-DCs were used as the negative control. All Mo-DCs were seeded onto six-well plates (5×10^5 cells per well) and cultured for the required incubation period.

2.4. Virus titration assay

SHXB- and UV-SHXB-infected immature Mo-DCs were seeded onto six-well plates (5×10^5 cells per well) and cultured for 2, 9, 24, 48 h. The virus was collected by freezing and thawing the plates three times, and determined by the tissue culture infectious dose 50 ($TCID_{50}$) in ST cells.

2.5. Quantification of SHXB strain M gene mRNA by real-time quantitative RT-PCR

Total RNA was extracted from approximately 5×10^5 of SHXB infected- and UV-SHXB treated-immature Mo-DCs harvested at 12, 24, 48 h p.i. with a commercial kit (TaKaRa, Japan). Then, RT-PCR was performed using an RT-PCR kit (TaKaRa) according to the manufacturer's instructions. To determine the gene expression profiles, individual samples were diluted 1:10 and 2 μL was amplified in a 20 μL reaction containing 10 μL of SYBR Premix™ Ex Taq (TaKaRa), 0.4 μL of ROX dye II and 0.4 μM of each of the forward and reverse gene-specific primers using an ABI 7500 instrument (Applied Biosystems, USA). The TGEV M gene was the target gene (GenBank accession no. *AF302262.1*), and the primers were: FP: gtgcttcctctcgaaggtgt, RP: cccatccagtcgcactactt. Porcine GAPDH gene was used as the internal parameter (GenBank accession no. *NM_001206359.1*). The primers used for this were: FP: tcatcatctctgccccttct, RP: gtcatgagtccctccacgat. The data were analysed using the ABI PRISM 7500 software tool (Applied Biosystems). Quantification of the target gene was determined by relative standard curves. TGEV M gene and Porcine GAPDH plasmids produced a double-standard curve; the Porcine GAPDH quantity at each sampling time was taken as the standard to determine the target genes quantity.

2.6. Detection of phenotype alteration of SHXB- and UV-SHXB-infected immature Mo-DCs by flow cytometric analysis

Mock, SHXB-infected, UV-SHXB-treated and LPS-treated immature Mo-DCs (1×10^5 cells) were respectively transferred to 1.5 mL centrifuge tubes at 24 h p.i., then washed with 0.01 M PBS twice, and incubated for 30 min at 4 °C with appropriate dilutions of the following primary antibodies: FITC-conjugated mouse anti-swine monoclonal antibody to swine histocompatibility leukocyte Ag II-DR (SLA-II-DR, Clone number: 2E9/13) (LifeSpan BioSciences, USA), FITC-conjugated mouse anti-human monoclonal antibody to the co-stimulatory molecules cluster of differentiation 80/86 (CD80/86, fusion protein Human CTLA-4, Clone number:B7-1/B7-2, Ancell Corporation, USA,), FITC-conjugated mouse anti-porcine monoclonal antibody to cluster of differentiation 1a (CD1a, Clone number: 76-7-4) (Abcam, Hong Kong), and PE-conjugated mouse anti-porcine monoclonal antibody to Swine Workshop Cluster 3a (SWC3a, Clone number: 74-22-15) (Abcam). Then, cells were washed twice with 0.01 M PBS. Finally, the cells were suspended in 200 μL PBS, 1×10^4 cells were analysed by flow cytometry (BD FACSVerse, BD, USA) and the results from flow cytometry were evaluated using the percentage of positive cells. Meanwhile, the isotype-matched mouse antibody (Abcam) was added as an isotype control.

2.7. Apoptosis assay

To quantify the apoptosis of immature Mo-DCs infected with the SHXB and UV-SHXB at 24 h p.i, the dual parameter analysis of Annexin V-FITC (Invitrogen) and Propidium iodide (Sigma) were performed. All samples (1×10^5 cells per sample) were labelled with both Annexin V-FITC (2 mg/mL) and PI (50 mg/mL) for 15 min. After washing three times with 0.01 M PBS, these cells were analysed by flow cytometry (BD FACSVerse, BD).

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