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Porcine monocyte-derived dendritic cells can be differentially activated by vesicular stomatitis virus and its matrix protein mutants

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ABSTRACT

Vesicular stomatitis virus (VSV) can cause serious vesicular lesions in pigs, and the matrix (M) protein is its predominant virulence factor. Dendritic cells (DCs) act as the bridge between innate and adaptive immune responses. However, the susceptibility of porcine DCs to VSV infection and the role of M protein in modulating the function of infected DCs are still poorly defined. Thus, this study aimed to determine the ability of virulent wild-type VSV(wtVSV) and two attenuated M protein variants ($VSV_{\Delta M51}$ and VSV_{MT}) to induce maturation of porcine monocyte-derived DCs (MoDCs) *in vitro*. It was found that both wtVSV and the M protein mutant VSVs could productively replicate in porcine MoDCs. Infection with wtVSV resulted in weak proinflammatory cytokine responses and interfered with DC maturation *via* downregulation of the costimulatory molecule complex CD80/86. Whilst $VSV_{\Delta M51}$ could activate porcine MoDCs, VSV_{MT}, a highly attenuated recombinant VSV with triple mutations in the M protein, induced a potent maturation of MoDCs, as evidenced by efficient cytokine induction, and upregulation of CD80/86 and MHC class II. Overall, our findings reveal that porcine MoDCs are differentially activated by VSV, dependent on the presence of a functional M protein. M protein plays a crucial role in modulating porcine DC-VSV interactions. The data further support the potential use of VSV_{MT} as a vaccine vector for pigs.

1. Introduction

Vesicular stomatitis virus (VSV) possesses a single-stranded negative-sense RNA genome and has a lytic life cycle; its natural hosts include livestock species, such as pigs and cattle. VSV infection can cause diseases involving vesiculation and ulceration of the tongue and oral epithelia, with occasional lesions on the feet and teats (Rose and Whitt, 2001). Five structural proteins are encoded by the VSV genome, including a nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and an RNA replicase (L) (Rose and Whitt, 2001). M protein is a multi-functional protein involved in virus assembly, budding, and pathogenesis (Harty et al., 1999; Irie et al., 2007), which can inhibit host gene expression at the level of transcription, nuclear-cytoplasmic transport, and translation (Ahmed and Lyles, 1998; Faria et al., 2005; Connor and Lyles, 2002). In our previous study, we found that pigs inoculated with wild-type VSV (wtVSV) developed serious vesicular lesions. In contrast, pigs inoculated with VSV carrying a mutated M protein, $VSV_{\Delta M51}$, developed mild lesions (Fang et al., 2015). Furthermore, infection with a recombinant VSV carrying triple mutations (ΔM51, V221F, and S226R) in the M protein (VSV_{MT}) did not result in vesicular stomatitis lesions, even in animals inoculated with a high dose. Notably, VSV_{MT} induced an effective immune response in pigs (Fang et al., 2015).

Dendritic cells (DCs) link the innate and adaptive immune systems and orchestrate the immune responses to pathogens (Idoyaga and Steinman, 2011). Infection of DCs by viruses can have a marked effect on these cells and important functional consequences for the generation of subsequent adaptive immune responses. VSV is an attractive candidate as a vaccine vector for delivery of heterologous antigens (Finke and Conzelmann, 2005). In this context, infection and activation of DCs would promote more effective T-cell responses to vector-expressed antigens. To date, the interactions between porcine DCs and VSV have remained largely unknown. Existing data mainly focus on VSV infection in rodent models. Ahmed et al. (2006) showed that wtVSV can interfere with murine DC maturation. However, mice are an unnatural host for VSV and there are clear differences between mice and pigs with regards to VSV infection (Martinez et al., 2003). Thus, in our study, we aimed to assess the susceptibility and functional consequences of porcine monocyte-derived DCs (MoDCs) infection with wtVSV and the two attenuated VSV variants $VSV_{\Delta M51}$ and VSV_{MT} (as a group, referred to as

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VSVs). We measured the viral infectivity and replication of the VSVs as well as cellular apoptosis, cytokine expression profiles, and modulation of cell surface proteins associated with DC maturation. Clarifying the susceptibility of porcine DCs to VSV and the role of the VSV M protein in modulating DC functions could help to understand pathogenesis of VSV, as well as support the development of attenuated VSV, such as VSV_{MT}, as a vaccine vector for pigs.

2. Materials and methods

2.1. Animals and viruses

Six-week-old healthy Bama minipigs were purchased from Swine Centre, University of Shanghai JiaoTong University, China. Animal experiments were conducted in accordance with the ethical guidelines of Shanghai JiaoTong University. The VSV_{IND}-based recombinant virus VSV_{XN2} and mutant VSV with methionine 51 deleted from the M protein ($VSV_{\Delta M51}$) were kept in our lab. Viral stocks were amplified by passage at low multiplicity of infection (MOI) of 0.01 in BHK21 cells. Recombinant viruses were concentrated by ultracentrifugation at 30,000 rpm/min for 2 h and frozen at −70∘C. Virus was titrated by plaque assay on Vero cells. VSV_{MT}, which carries three mutations in its M protein (ΔM51, V221F, and S226R), was prepared as described previously (Fang et al., 2015). VSV_{XN2} was used as the wtVSV in the study. In selected experiments recombinant WT and mutant VSV strains expressing enhanced green fluorescence protein (eGFP) (VSV-GFP, $VSV_{\Delta M51}$-GFP, and VSV_{MT}-GFP) were used (Fang et al., 2015).

2.2. Isolation of porcine monocytes and generation of monocyte-derived DCs

Porcine blood was collected by heart puncture using collagen-coated syringes and mononuclear cells were isolated using a HistoPaque 1.077 gradient reagent (Sigma, USA). Monocytes were isolated using anti-human CD14 microbeads (Miltenyi Biotec, USA) and an LS column according to the manufacturer's instructions. To generate porcine MoDCs, monocytes were cultured in RPMI-1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) plus 10 ng/ml of recombinant porcine interleukin (IL)-4 (R&D, USA) and 10 ng/ml of granulocyte–macrophage colony-stimulating factor (GM-CSF) (R&D Systems, USA) for 7 days at 37 °C with 5% CO_2 (Facci et al., 2010). Half of the medium was replaced every three days. The non-adherent MoDCs were harvested, washed, counted, and used in subsequent assays.

MoDCs and monocytes were characterized by flow cytometry. Briefly, mouse anti-pig MHCII (Clone 2E9/13, BioRad Antibodies, USA) was labeled with phycoerythrin (PE) using a Zenon mouse IgG2a labeling kit (Invitrogen, USA). CD80/86 was detected with a human CD152-muIg fusion protein conjugated with allophycocyanin (APC) (Ancell, USA). Immunofluorescence staining was performed by incubating 1×10^5 cells with each reagent for 20 min at room temperature. Cells were washed three times and fixed with 2% paraformaldehyde. 5000 events were collected using a CytoFlex flow cytometer (Beckman Coulter, USA) and analyzed by CytExpert 1.2 software (Beckman Coulter). Mouse IgG2a-PE and IgG2b-APC isotype antibodies were used as controls (Southern Biotech, USA).

2.3. Replication curves of VSVs in porcine myeloid cells

Kinetic studies were conducted for the analysis of virus replication. In short, MoDCs or monocytes were infected with wtVSV, $VSV_{\Delta M51}$, or VSV_{MT} at a multiplicity of infection (MOI) of 1. After 1 h of absorption, the inoculum was removed, and the cells were washed three times with Dulbecco's phosphate-buffered saline (DPBS), and RPMI-1640 medium supplemented with 2% FBS was added. The infected cells were incubated at 37 °C, and triplicate aliquots of cell culture supernatant were removed at 2, 8, 12, 24, and 48 h post-infection (hpi). Yields of progeny virus were determined by a plaque assay in Vero cells as previously described (Fang et al., 2015).

2.4. Apoptosis assay

1×10^5 MoDCs were grown in 96-well tissue culture plates and infected with VSV-GFP, $VSV_{\Delta M51}$-GFP, or VSV_{MT}-GFP at an MOI of 10. Phosphatidylserine exposure was determined using an apoptosis detection kit (BD Biosciences, USA), according to the manufacturer's protocol. In brief, cells were harvested at the indicated times of 0, 4, 8, or 12 hpi, washed with cold DPBS, and suspended in 100 µl of annexin-binding buffer. The cells were then incubated with PE-conjugated annexin V and 7-AAD at room temperature (RT) for 15 min in the dark. Following the incubation period, 400 µl of annexin-binding buffer was added to each sample, and the samples were mixed gently and kept on ice. The VSV-infected cells were quantified using flow cytometric detection of the eGFP reporter expression and were gated out for the apoptosis assay. The fluorescent signals of annexin V-PE and 7-AAD were detected using a CytoFlex flow cytometer. UV-inactivated wtVSV was used as a mock infection control.

2.5. Western blot analysis

For caspase-mediated apoptosis marker detection in MoDCs, 5×10^5 cells were grown in 24-well plates and infected with wtVSV, $VSV_{\Delta M51}$, or VSV_{MT} at an MOI of 10. Immunoblots were probed with an antibody against human caspase-3 (Cell Signaling Technology, USA) at a dilution of 1:1000. Detection of porcine β-actin protein was used to normalize sample loading. Primary monoclonal antibody detecting β-actin (Santa Cruz, USA) was diluted 1:1000. Horseradish peroxidase-conjugated goat anti-mouse IgG, used as the secondary antibody, was diluted 1:5000 (Santa Cruz, USA). Protein bands were observed using West Pico Chemiluminescent Substrate (Thermo Scientific, USA) and exposed to Kodak BioMax MR film (Kodak, USA). Target protein bands were scanned and analyzed, which normalized the amounts to β-actin. Quantification of target protein intensity relative to β-actin was calculated using Image J software by NIH as follows: (intensity of protein X/intensity of β-actin) × 100. UV-inactivated wtVSV was used as a mock infection control.

For detecting viral structural protein expression in monocytes, 5×10^5 cells were grown in 24-well plates and infected with wtVSV, $VSV_{\Delta M51}$, or VSV_{MT} at an MOI of 10. Cells were harvested at the indicated times of 0, 4, 8, 12, or 24 hpi, washed with cold DPBS, and then solubilized in RIPA buffer containing 1 mM phenylmethylsulfonyl fluoride, 1 mM aprotinin, and 1 mM pepstatin. Proteins were resolved by SDS-polyacrylamide gel electrophoresis (PAGE) on 10% polyacrylamide gels. Following electrophoresis, gels were electroblotted onto polyvinylidene difluoride membranes and blocked in Tris-buffered saline (pH 7.5) containing 5% non-fat dry milk. Typical bands for VSV proteins were detected using convalescent sera from wtVSV-infected mice at a dilution of 1:2000. Horseradish peroxidase-conjugated goat anti-mouse IgG, used as the secondary antibody, was diluted at 1:5000 (Santa Cruz, USA). Protein bands were also observed using West Pico Chemiluminescent Substrate (Thermo Scientific, USA) and exposed to Kodak BioMax MR film (Kodak, USA). Viral protein bands were scanned, and the protein intensity relative to β-actin was calculated as described above.

2.6. Cytokine assays

Levels of cytokines, including TNF-α, IL-1β, IL-6, IL-8, IL-12p40, and IL-4, as well as type I interferon (IFN) bioactivity were detected in the supernatants of VSV-infected MoDC and monocyte cultures. Porcine embryo fibroblasts (PEFs) were prepared as previously described (Zhang et al., 2006) and set up as the 'non-antigen-presenting cell' control. In short, 1×10^5 MoDCs, monocytes, or PEF cells were