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Research paper

Monocyte-derived dendritic cells enhance cell proliferation and porcine circovirus type 2 replication in concanavalin A-stimulated swine peripheral blood lymphocytes *in vitro*

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ABSTRACT

Dendritic cells (DCs) are professional antigen presenting cells cooperating with other immune cells for the activation of innate and adaptive immune responses. The objective of the present study was to investigate the replication activity of porcine circovirus type 2 (PCV2) in DCs and/or lymphocytes during their cross talk and its possible mechanism. Two models were set, herein. Swine blood monocyte (Mo)-derived DCs (MoDCs) or peripheral blood lymphocytes (PBLs) were inoculated with PCV2 prior to their co-cultivation. Bacterial lipopolysaccharide (LPS) and concanavalin A (Con A) were used to stimulate MoDCs and PBLs, respectively. During 6 days of cultivation, a high PCV2 antigen-containing rate without detectable intranuclear signals and a slight but significant increase in the copy number of PCV2 genome were detected in PCV2-inoculated MoDCs. The presence of LPS alone or PCV2-free PBLs, however, had no effect on the location of PCV2 antigens or copy number of PCV2 genome in PCV2-inoculated MoDCs. On the contrary, active PCV2 replication occurred in Con A-stimulated PCV2-inoculated PBLs. When compared with blood Mos, MoDCs induced significantly higher cell proliferation and intensified PCV2 replication in Con A-stimulated PCV2-inoculated PBLs, for which direct contact between MoDCs and lymphocytes was required. Among the cytokines secreted by Con A-activated PBLs, interleukin (IL)-2, but not IL-4 or interferon-γ, could induce cell proliferation and PCV2 replication in PCV2-inoculated PBLs. The findings suggest that although MoDCs support only limited PCV2 replication in themselves, their accessory cell function is required for cell proliferation and PCV2 replication in PCV2-infected lymphocytes.

Abbreviations: ANOVA, analysis of variance; APCs, antigen presenting cells; CFSE, 5-(and 6)-carboxyfluorescein diacetate succinimidyl ester; Con A, concanavalin A; DCs, dendritic cells; DMEM, Dulbecco modified Eagle medium; Ds, double-stranded; DPI, days post-inoculation; DPC, days post-initiation of co-culture; ED50, 50% effective dosages; FITC, fluorescein isothiocyanate; FSC, forward scatter; HIFBS, heat-inactivated fetal bovine serum; IFA, immunofluorescence assay; IFN-γ, interferon-gamma; IHC, immunohistochemical; IL-2, interleukin-2; IL-4, interleukin-4; ISH, *in situ* hybridization; LPS, lipopolysaccharide; MLCs, monocyte-macrophage lineage cells; MoDCs, monocyte-derived dendritic cells; Mos, monocytes; PBLs, peripheral blood lymphocytes; PCV2, porcine circovirus type 2; PK-15, porcine kidney-15; PRRSV, porcine reproductive and respiratory syndrome virus; RPE, R-phycoerythrin; rt-PCR, real-time PCR; SSC, side scatter; SPF, specific pathogen free; TLR, toll-like receptor.

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1. Introduction

Porcine circovirus type 2 (PCV2) is a small, non-enveloped, single-stranded, circular DNA virus (Meehan et al., 1998; Tischer et al., 1982), and the infection is distributed in pig herds worldwide (Segales et al., 2005). Experimental and field studies indicate that PCV2 is a required but insufficient causative agent of postweaning multisystemic wasting syndrome (PMWS), a disease that has a significant impact on swine production. Currently, although the majority of weaned pigs in the field carry PCV2, only some will develop PMWS (Darwich et al., 2004; Segales et al., 2005). A significantly higher tissue load of PCV2 antigen and/or nucleic acid is one of the characteristic features of PMWS (Chianini et al., 2003; Lin et al., 2010). Many infectious and non-infectious factors are recognized to trigger PCV2 replication and determinate the clinical outcome of PCV2 infection (Darwich et al., 2004; Segales et al., 2005; Tomas et al., 2008).

Previous *in vitro* and *in vivo* studies have shown that PCV2 antigen and/or nucleic acid can accumulate to a high level in the cytoplasm of monocyte (Mo)-macrophage lineage cells (MLCs) (Chianini et al., 2003; Lin et al., 2010), including dendritic cells (DCs) (Vincent et al., 2003). However, replication of PCV2 in cultured MLCs is not recognized as a dominant feature (Chang et al., 2006b; Fernandes et al., 2007; Vincent et al., 2003). Although the physiological role of DCs in peripheral tissue is to bring antigen to regional lymph nodes and present them in a proper form to T-lymphocytes (Granucci et al., 2004), there is a lack of evidence that PCV2-containing DCs would have their cell surface markers modulated, transmit PCV2 to syngeneic T lymphocytes, or induce death of co-cultured lymphocytes under the *in vitro* culture system (Vincent et al., 2003). It is also generally considered that the relatively simple structure makes PCV2 uncapable of actively modifying the function of DCs as other more complex swine viruses do (McCullough et al., 2009). Meanwhile, it has been demonstrated that PCV2 DNA could inhibit cytokine secretion by plasmacytoid DCs stimulated with toll-like receptor (TLR) ligands (Vincent et al., 2007). Particularly, the double-stranded (ds) DNA of the replicative form of PCV2 has been shown to have a high immunomodulatory capability, such as interfering in the cytoskeletal rearrangement and endocytic process of DCs (Balmelli et al., 2011). Therefore, the structure of PCV2 contained in DCs has been mainly implicated for the insufficient recognition of other viral and bacterial danger signals and favoring the establishment of other infections (Balmelli et al., 2011; McCullough et al., 2009; Vincent et al., 2007).

Unlike MLCs, only limited PCV2 antigen and/or nucleic acid could be detected in lymphoid cells by *in situ* hybridization (ISH), immunohistochemical (IHC) staining, or immunofluorescence assay (IFA) (Darwich et al., 2004; Yu et al., 2007a). However, PCV2 could replicate in lymphoid cells, including B and T cells (Yu et al., 2007a,b). Mitogen-stimulated peripheral blood lymphocytes (PBLs) have been used as an *in vitro* model to investigate PCV2 replication under immune activation (Lefebvre et al., 2008; Lin et al., 2008; Yu et al., 2007b). Various mitogens, including concanavalin A (Con A), are capable of inducing immune cell activation, resulting in an array of cytokine production (Verfaillie et al., 2001) and ultimate proliferation of the activated T cells. The mechanism of mitogenicity is complicated and its correlation with PCV2 infection is still not clear. In human and mouse, cellular interaction and interleukin 2 (IL-2) are the two requirements for T lymphocyte colony formation (Austyn et al., 1983; Woods and Lowenthal, 1984). It is known that antigen presenting cells (APCs) provide co-stimulatory signals necessary for the proliferation of T lymphocytes (Austyn et al., 1983; Van Voorhis et al., 1983). Monocytes, macrophages, DCs, B lymphocytes, and a portion of T lymphocytes all possess this ability, but the capability varies (Basta et al., 2000). Dendritic cells are professional APCs with an outstanding ability to co-stimulate naive T lymphocytes (Croft et al., 1992) and subsequently direct the quality of the adaptive immune response.

It is now agreed that PCV2 is monocytotropic (Chang et al., 2006a,b; Chianini et al., 2003) as well as lymphocytotropic (Yu et al., 2007a,b). The questions are whether PCV2 replicates in APCs and/or lymphocytes during their cross talk and what host factors facilitate PCV2 replication. In the present study, two major experimental models of MoDC and PBL co-culture were conducted to clarify these issues. Information obtained from the study will bring us a further step closer to understanding the features of PCV2 infection during the onset of adaptive immune response.

2. Materials and methods

2.1. Experimental animals

Three 5-week-old, crossbred, male and female, cesarean-derived, colostrum-deprived, primary specific-pathogen-free (SPF) piglets from the Animal Technology Institute Taiwan were used for periodic blood collection. These pigs were tested free of the common swine viral and bacterial pathogens as listed previously (Lin et al., 2008) and kept in a laboratory animal facility with an isolated air conditioning system. During the study, regular examination of blood samples for porcine reproductive and respiratory syndrome virus (PRRSV) and PCV1/PCV2 nucleic acids and antibodies was performed to ensure freedom of these viruses. All study procedures and animal care were conducted in accordance with the guidelines and under the supervision of the National Taiwan University Institutional Committee on Animal Care and Use.

2.2. Viruses

The isolate of PCV2 and the method used for viral propagation were the same as previously described (Chang et al., 2006a). The PCV2 stock had a titer of 5×10^6 $TCID_{50}$/ml as titrated in PK-15 cell line which contained $10^{9.32}$ copies of PCV2 genome/ml as quantified by real time PCR. The genomic size of the PCV2 isolate was 1768 bp and completely matched with the one in GenBank, AY146993.