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Porcine circovirus type 2 (PCV2) distribution and replication in tissues and immune cells in early infected pigs

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Abstract

Replication of porcine circovirus type 2 (PCV2) in pigs, as measured by spliced capsid mRNA (Cap mRNA) and viral DNA, was investigated following experimental infection. Peripheral blood mononuclear cells (PBMCs), and tissue from bronchial lymph nodes (BLN), inguinal lymph nodes (ILN), tonsils, lungs, liver, kidneys, spleen and thymus from infected pigs on different days post-infection (DPI) were assessed. PCV2 replication differed dramatically between tissues from the same infected pig. The virus actively replicated in most tested tissues at 14 DPI in association with increased PCV2 associated lesions and PCV2 antigen levels, although no clinical signs correlated with PCV2 associated disease were observed in infected pigs during the course of the study. The PCV2 Cap mRNA was detected only at 13 DPI in PBMCs from infected pigs, suggesting replication of the virus in circulating blood is transient and not a major site for PCV2 replication *in vivo*. Evaluation of the Cap mRNA and viral DNA synthesis in T and B lymphocyte and monocyte populations from PBMCs and BLN at various intervals post-inoculation revealed replication of PCV2 in all cell subpopulations; however, viral replication in B lymphocytes was greater than observed in mononuclear cells isolated from BLN at 14 DPI indicating that B lymphocytes may be an important cell population for PCV2 replication. These findings further our understanding of the cell types permissive for PCV2 replication and the pathogenesis of PCV2 infection *in vivo*.

Keywords: PCV2; Real-time RT-PCR; *In vivo*

1. Introduction

Porcine circovirus type 2 (PCV2) is a circular single-stranded DNA virus in the Circoviridae family (Tischer et al., 1982; Brunborg et al., 2004; Meehan et al., 1998). It was first associated with pigs exhibiting postweaning multisystemic wasting syndrome (PMWS) in Western Canada (Clark, 1997). Depletion of lymphocytes in the lymphoid follicles and their replacement by macrophages are the hallmark lesions observed in this syndrome (Allan et al., 1999; Meehan et al., 1998). Infection with PCV2 has also been associated with a number of other pathologic disease syndromes including enteritis, pneumonia and respiratory disease, abortion and porcine dermatitis and nephropathy syndrome (PDNS) (Allan and Ellis, 2000; Segales et al., 2004).

In situ hybridization (ISH) and polymerase chain reaction (PCR) assays have been used to detect PCV2 DNA in tissues and cells from infected pigs. In addition, the lymphocyte depletion of follicular and interfolli-

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cular lymphoid tissues together with the macrophage infiltration of lymphoid tissues observed in PMWS affected pigs are highly correlated with a decrease in numbers of circulating B and T cells and reduced numbers of lymphocytes in lymphoid organs (Chianini et al., 2003; Clark, 1997; Darwich et al., 2002; Nielsen et al., 2003; Quintana et al., 2001; Rosell et al., 1999; Sarli et al., 2001; Segales et al., 2000; Shibahara et al., 2000). However, it is not clear which tissue or cell type is the primary site for PCV2 infection and replication. Recently we demonstrated that PCV2 replicates in PBMCs using a quantitative real-time reverse transcription-PCR (RT-PCR) assay that detects PCV2 spliced capsid mRNA (Cap mRNA) and allows discrimination between replicating PCV2 and PCV2 virions (Yu et al., 2004). The aim of this study was to understand the replication of PCV2 early in infection *in vivo* and identify the permissive cell populations. In this study, we investigated the levels of PCV2 Cap mRNA and viral DNA in PBMCs, bronchoalveolar lavage (BAL) cells, and multiple tissues (bronchial lymph nodes (BLN), inguinal lymph nodes (ILN), lung, tonsil, thymus, spleen, liver and kidney) from pigs during the acute phase of PCV2 infection as well as the viral DNA levels in serum and cell-free BAL fluid. The microscopic lesions and the amount of PCV2 antigen in tissues were measured using histopathology and immunohistochemistry (IHC), respectively. PCV2 antibody levels in serum and BAL fluid were also assessed. Specific immune cell populations were sorted by AutoMACS from PBMCs or BLN mononuclear cells and the PCV2 infection and replication in each cell population was further measured by real-time RT-PCR and real-time PCR assays, respectively. Our studies show that PCV2 replication differs between tissues from the same pig and demonstrates that lymphocytes are permissive for PCV2 replication while monocytes may be the site for PCV2 persistence in infected pigs.

2. Materials and methods

2.1. PCV2 inocula

The challenge inoculum was prepared from a PCV2 virus stock derived from the molecular clone of PCV2 isolate 40895 (GenBank accession no. AF264042). This virus was isolated from the spleen of a pig with naturally occurring PMWS (Fenaux et al., 2002). The titer of the PCV2 inoculum was $1 \times 10^{4.75}$ 50% tissue culture infective dose ($TCID_{50}$)/ml as determined by titration on PCV-free PK-15 cells using a previously described immunofluorescence assay (IFA) (Fenaux et al., 2002).

2.2. Experimental design

Twenty-four colostrum-fed, cross-bred conventional pigs with low maternal antibody titers against PCV2 were used in this study (Opriessnig et al., 2004a,b). The pigs were assigned to two rooms (twelve pigs/room) on the day of challenge (trial day 0). Twelve pigs in one room were inoculated intranasally at the age of 4–5 weeks with 5 ml of the PCV2 inoculum. The remaining pigs served as negative controls were housed in a separate room and sham-inoculated with 5 ml of PCV-free PK-15 cell lysate. All study procedures and animal care were conducted in accordance within the guidelines and under the supervision of the Iowa State University Institutional Committee on Animal Care and Use.

2.3. Necropsy and sample collection

Three pigs from each group were necropsied at 3, 7, 14 and 21 days post-infection (DPI). Tissue samples from BLN, ILN, lungs, tonsils, thymus, spleen, liver and kidney of each pig were collected at necropsy and fixed in 10% formalin for histological examination and IHC assay. Fresh BLN samples were also placed immediately in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin for isolation of mononuclear cells. In addition, pieces of each tissue described above were snap frozen on dry ice and stored at −80 °C until used for isolation of total RNA or DNA.

The right lung of each necropsied pig was lavaged with 50 ml of collecting solution (sterile phosphate-buffered saline (PBS) with 1% bovine serum albumin (BSA), 300 U/ml penicillin and 300 mg/ml streptomycin). The BAL was centrifuged (400 × *g*, 10 min, 4 °C) to separate cells and cell-free lavage fluid. BAL cells (1×10^6) were resuspended in 200 μl RNAlater (Ambion, Austin, TX) for total RNA isolation and an additional 1×10^6 viable cells were placed in 200 μl PBS for DNA isolation. The cell-free lavage fluid was stored at −20 °C for PCV2 antibody detection and DNA isolation. Serum samples were collected at 0, 2, 6, 13, and 20 DPI and stored at −20 °C until used for DNA isolation or assayed for PCV2 antibodies. Concurrently, heparinized blood samples were collected for PBMC isolation.

2.4. Isolation of mononuclear cells from BLN and peripheral blood

Fresh BLN tissues were cut into small pieces and pushed through a 70 μm pore-size nylon cell strainer (Falcon) to create single-cell suspensions. The cells