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Systemic cytokine profiles of mice vaccinated with naked DNAs encoding six open reading frame antigens of porcine circovirus type 2 (PCV2)

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Abstract

The objective of this study was to characterize the murine immune response to vaccination with DNAs encoding each of six porcine circovirus type 2 (PCV2) open reading frames (ORFs). After intramuscular vaccination with the naked DNAs, blood was taken on days post-infection (DPI) 7 and 35 and subjected to Bio-Plex cytokine assays. ORF3 elicited high levels of the pro-inflammatory cytokine TNF-α ($P < 0.001$) and decreased IL-12 levels ($P < 0.001$) on DPI 7, and was lethal for nine of the 15 vaccinated mice, which died on DPIs 4, 6, 9, and 10. The remaining six mice showed general prostration but then recovered. The clinical signs correlated with the systemic TNF-α and IL-12 levels. ORF1 vaccination elevated T-helper (Th)1 (IFN-γ; $P < 0.001$) and Th2 (IL-13; $P < 0.05$) cytokine levels on DPI 35, while ORF2 markedly elevated the expression of the humoral immunity- and Th-2-related cytokine IL-10 ($P < 0.001$) on DPI 35. These observations provide insights into the immune responses generated by each PCV2 ORF.

Keywords: Cytokine profile; PCV2; ORFs

1. Introduction

Porcine circovirus type 2 (PCV2) is associated with various disease syndromes in pigs, including postweaning multisystemic wasting syndrome (PMWS), proliferating and necrotizing pneumonia (PNP), porcine dermatitis and nephropathy syndrome (PDNS), porcine respiratory disease complex (PRDC), and abortion and reproductive problems. These syndromes are now grouped under the condition porcine circovirus disease (PCVD) (Allan and Ellis, 2000). It appears that at least some of these diseases arise because PCV2 impairs the immune system. For example, the development of PMWS has been associated with aberrant stimulation of the immune system and immunosuppression (Segales et al., 2001; Allan et al., 2000; Krakowka et al., 2001) characterized in part by the loss of peripheral B and T cells (Segales et al., 2001; Nielsen et al., 2003). Moreover, the lymphoid tissues (Kim and Chae, 2004; Darwich et al., 2003b) and peripheral blood mononuclear cells (PBMCs) (Darwich et al., 2003a) of pigs with PMWS showed increased mRNA expression of cytokines and chemokines that are involved in the recruitment of inflammatory cells to injured tissue, namely, MIP-1, MCP-1, IL-1β, IL-8, and TNF-α.

The two known types of PCVs, which are referred to as nonpathogenic PCV and PMWS PCV, both contain 11 potential ORFs (Hamel et al., 1998). The *rep* (ORF1) gene is essential for viral replication (Cheung, 2003; Mankertz et al., 2004) while the cap (ORF2) gene encodes the viral capsid, which is the major structural protein of the virus. In addition, transfection of cells with ORF3 alone induces

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apoptosis *via* a pathway that is similar to that induced by PCV2 infection (Liu et al., 2006).

Vaccination with plasmid DNA encoding foreign antigens is one of the most significant advances that has been made in immunology and vaccinology in recent years (Fynan et al., 1993; Ulmer et al., 1996; Wolff et al., 1990). It has been shown that administration of the DNA by intramuscular immunization generally elicits cellular (Th1) immune responses, whereas particle bombardment by using a gene gun predominantly elicits antibody (Th2) responses (Sasaki et al., 2003). For example, a previous study has shown that when mice were injected with PCV2 open reading frame 2 (ORF2) by using a gene gun, they produced high levels of antibodies against PCV2 (Kamstrup et al., 2004). Here, we investigated the cellular immune responses generated by vaccinating mice intramuscularly (i.m.) with high concentration of each of the different PCV2 ORFs and then examining the function of ORFs and the cytokine profiles in their PBMCs seven and 35 days later.

2. Materials and methods

2.1. Generation of naked DNAs encoding ORFs1-6

The specific PCV primers used for gene cloning were designed on the basis of the complete genome sequence of the Korean PCV2 isolate (06Q001-1), which was obtained in 2006 (An et al., 2007). The sense primers for ORFs1-6 included the initial codon and each bore an EcoRI enzyme site (GAATTC). The antisense primers for ORFs1-6 contained the stop codon and each bore an XbaI enzyme site (TCTAGA) (Table 1). The ORF1-6 primers amplified a 945, 700, 314, 179, 179, and 116 bp fragment, respectively.

The PCR amplified fragments were then eluted and cloned into a pGEM-T plasmid (Promega, USA), after which the cloned ORFs were digested with EcoRI and XbaI (ORF1, ORFs3-6) or with NotI (ORF2) in the multicloning site of pGEM-T and recloned into PCI mammalian expression vector (Promega, USA). The ORF-encoding PCI plasmids were extracted by using the Qiagen Plasmid Giga Kit (Qiagen, Germany) and used as the naked DNA vaccine. The purity of the naked DNA vaccine was determined by the ratio of the absorbance at 260 nm divided by the reading at 280 nm. Good-quality DNA with A_{260}/A_{280} ration of 1.7–2.0 was used for injection of mouse.

2.2. Injection of mice with ORF-encoding naked DNAs

Six groups of fifteen female BALB/c mice (eight-week-old) were injected i.m. with a syringe containing 0.5 mg/100 μl ORF-encoding naked DNA. As a control, a group

Table 1
Primers and probes used in this study

ORF	Primer & Probe sequences
ORF1 Sense	5′- GAATTCATGCCCAGCAAGAAGA-3′
ORF1 Antisense	5′- TCTAGATCAGTAATTTATTTCA-3′
ORF2 Sense	5′- GAATTCATGACGTATCCAAGGAG-3′
ORF2 Antisense	5′- TCTAGATTAGGGGTTAAGTGGGG-3′
ORF3 Sense	5′- GAATTCATGGTAACCATCCCACCAC-3′
ORF3 Antisense	5′- TCTAGATTACTTATTGAATGTGGAG-3′
ORF4 Sense	5′- GAATTCATGACGTGTACATTGGTCT-3′
ORF4 Antisense	5′- TCTAGATCAAGGACAACGGAGTGAC-3′
ORF5 Sense	5′- GAATTCATGTACACGTCATTGTGGG-3′
ORF5 Antisense	5′- TCTAGATCAGTAGATCATCCCACGG-3′
ORF6 Sense	5′- GAATTCATGGTTTTTATTTTTCATT-3′
ORF6 Antisense	5′- TCTAGACTACAAACTGCTGGAAATG-3′
ORF1 Real-Time Sense	5′- GCGAGGAGGGTAATGAGGA -3′
ORF1 Real-Time Antisense	5′-CTGCAATATTCTTTATTCTGCTG-3′
ORF1 TaqMan probe	5′-56-FAM- TTGGGTGCCCGCTGCCACATCGAGAAAG –3BHQ-1-3′
ORF2 Real-Time Sense	5′- AAGGAAAAATGGCATCTTCAACAC-3′
ORF2 Real-Time Antisense	5′- TCCTGGGGGAAGAAAGTCA-3′
ORF2 TaqMan probe	5′-56-FAM- TCCTGGGCGGTGGACATGATGAGATTCA-3BHQ-1-3′
ORF3 Real-Time Sense	5′- ATCCCACCACTTGTTTCTAG -3′
ORF3 Real-Time Antisense	5′- GAAAATGCAGAAGCGTGATTGG -3′
ORF3 TaqMan probe	5’-56-FAM- ACCACACCCAGGTGGCCCCACAATGA-3BHQ-1-3′
ORF4 Real-Time Sense	5′- CGTGTACATTGGTCTTCCA -3′
ORF4 Real-Time Antisense	5′- CAACGGAGTGACCTGTCTA -3′
ORF4 TaqMan probe	5′-5-TET- ACAGGGTGCTGCTCTGCAACGGTCA-3BHQ-1-3′
ORF5 Real-Time Sense	5′- CTGGGTGTGGTAAAAGCAA -3′
ORF5 Real-Time Antisense	5′- GCCAGCCATAAAAGTCATCA -3′
ORF5 TaqMan probe	5′-5-TET- AGTGGTGGGATGGTTACCATGGTGAAGAAG-3BHQ-1-3′
ORF6 Real-Time Sense	5′- TCATTTAGGGGTTAAGTGGG -3′
ORF6 Real-Time Antisense	5′- CAAACTGCTGGAAATGTAGA -3′
ORF6 TaqMan probe	5′-56-FAM- TTTCGAACGCAGTGCCGAGGCCTAC-3BHQ-1-3′

The primers used for cloning encompass the outermost 5′- and 3′-ends of the ORFs. The start and stop codons are underlined.
The probes used for real-time PCR were labeled on their 3′-ends with BHQ and on their 5′-ends with FAM or TET.