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Immune gene expression profiles in swine inguinal lymph nodes with different viral loads of porcine circovirus type 2

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ABSTRACT

Porcine circovirus type 2 (PCV2) infection has been suggested as an acquired immunodeficiency disorder. However, the immunopathogenesis of PCV2 infection is still not fully clarified. In the present study, 35 inguinal lymph nodes (LNs) with different levels of PCV2 load obtained from postwaening multisystemic wasting syndrome (PMWS)-affected pigs and 7 from healthy subclinically PCV2-infected pigs were selected. The LNs were subsequently ranked by their PCV2 loads to mimic the progression of PCV2 infection-associated lesion development. The expressions of 96 selected immune genes in these LNs were assessed by the integration of several reverse transcription quantitative real-time polymerase chain reaction experiments. Hierarchical cluster analysis of the gene expression profiles resulted in 5 major clusters (A, B, C, D, and E). Different clusters of immune gene expression profiles were compatible with the divergent functions of various immune cell subpopulations. 61 out of 96 selected genes belonged to cluster C and were mainly involved in the activation of dendritic cells and B and T lymphocytes. The expression levels of these genes were generally up-regulated in the LNs obtained from PMWS-affected pigs with relatively lower PCV2 loads. However, the up-regulated level tended to reduce or turned into down-regulation as the PCV2 load increased. Genes belonging to cluster B, involved in T cell receptor signaling, became silenced as the PCV2 load increased. The expression profiles of macrophage-associated genes were either independent from or positively correlated with the PCV2 load, such as those in clusters A and E and in cluster D, respectively. In addition, the principle component analysis of the expression of the 96 selected genes in the 42 inguinal LNs revealed that 53.10% and 72.29% of the total data variants could be explained by the top-3 and top-7 principle components, respectively, suggesting that the disease development of PCV2 infection may be associated with a few major and some minor factors. In conclusion, assessment of immune gene expression profiles in LNs supports a close interaction between immune activation and suppression during the progression of PMWS development.

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 of PCV2 is widely distributed in pig herds worldwide (Segales,

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2012). Among various PCV2-associated diseases, post-weaning multisystemic wasting syndrome (PMWS) has the major economic impact on swine production (Opriessnig et al., 2007; Segales, 2012). High levels of PCV2 antigens/nucleic acid in the lymphoid system along with variable lymphoid depletion and granulomatous inflammation are the characteristic features of PMWS (Chianini et al., 2003; Sarli et al., 2001). The severity of lymphoid lesion correlates well with the PCV2 load of tissue, suggesting that PCV2-induced immune dysfunction may play a central role in the pathogenesis of PMWS (Chianini et al., 2003; Krakowka et al., 2005; Lin et al., 2011b).

PCV2 is considered as an immunosuppressive agent in pigs (Opriessnig et al., 2007; Segales, 2012). Over the past decade, several mechanisms of the lymphoid depletion observed in PMWS-affected pigs have been proposed. It has been suggested that lymphocyte depletion may be mediated by the up-regulation of apoptosis (Shibahara et al., 2000), cytokine imbalance (Darwich et al., 2003b), and alteration in cell migration (Sarli et al., 2001). The severe immune suppression caused by PCV2 infection has also been explained by the decrease in lymphocyte proliferation due to reduced or lack of production of growth factors (Mandrioli et al., 2004). More recent studies have pointed out that PCV2 may affect the immune system via the induction of IL-10 (Doster et al., 2010) and/or the down-regulated dendritic cell (DC) functions by its genome (Vincent et al., 2007). On the contrary, activation of the immune system, regardless triggered by co-infection and/or vaccination, is considered as a pivotal event in the induction of PMWS (Krakowka et al., 2001). The occurrence of granulomatous lymphadenitis represents a status of persistent inflammation in PMWS-affected pigs. However, information regarding the linkage between the original stimulus and chronic inflammation during the development of PMWS is rather limited. To date, emerging evidences support that chronic inflammation may contribute directly to the progression toward immune suppression in the context of illnesses, such as cancer and chronic infection (Baniyash, 2006; Blume et al., 2011). Lymphoid depletion and granulomatous inflammation are two characteristic but incompatible lesions concurrently present in PMWS-affected pigs, which may be a valuable model for studying the correlation between immune suppression and chronic inflammation.

Inferences based upon the immune-related gene expression profiles in LNs obtained from naturally PMWS-affected pigs are useful in understanding the pathogenesis of PMWS at the molecular level. By far, several microarray studies have demonstrated that the majority of genes affected following PCV2-inoculation (Lee et al., 2010; Tomas et al., 2010) or PMWS-development (Fernandes et al., 2012) are immune-related. However, pigs inoculated with PCV2 alone are usually asymptomatic with minimal lymphoid lesions (Lee et al., 2010; Tomas et al., 2010). It is also difficult to interpretate a large-scale of microarray-generated data accurately, in particular when the cell composition changes drastically and significant sample variations resulting from multiple factors occur during the development of PMWS (Fernandes et al., 2012). In fact, validation of microarray-generated data by reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) is usually required (Fernandes et al., 2012; Lee et al., 2010; Tomas et al., 2010). In addition to the standard expression monitoring arrays, there are also focused arrays and real-time PCR arrays contain only probe/primer sets against a small selection of genes representing a specific function or pathway (VanGuilder et al., 2008). As the number of genes relevant for a certain biological experiment is highly enriched, it in turn reduces the data complexity and the number of false positive findings (Gohlmann and Willem, 2009). In the present study, the measurement of changes in the expression profiles of a specified panel of immune genes, rather than whole genome, by several individual RT-qPCR experiments was, therefore, designed; this approach may serve as an alternative, but potentially more practical strategy to characterize the natural PCV2 infection-associated lymphoid lesions at the transcriptional level.

2. Materials and methods

2.1. Experimental design

To effectively investigate the feature of natural PCV2 infection-associated lymphoid lesions at the transcriptional level, the present study was, thus, designed to specialize on the panel of immune genes and conducted with a sufficient number of tissue samples of inguinal LNs obtained from the field. Through the procedure of sample collection and selection as described in Sections 2.2 and 2.3, 42 selected inguinal LNs were ranked by their PCV2 loads to mimic the progression of PCV2 infection-associated lesion development in LNs. A total of 96 genes were targeted for analysis, which included a panel of genes listed in a commercial human T cell and B cell activation real-time PCR array (QIAGEN, http://www.sabiosciences.com/rt_pcr_product/HTML/PAHS-053A.html) and/or described in the text book (Tizard, 2009). These genes are important for immune cell activation, maturation, and differentiation, including cytokines, chemokines, cell surface receptors, and their downstream signal transduction molecules. The details of the selected genes and the correspondent primer sets are listed in supplementary Table 1. The patterns of gene expression in the LNs were measured by SYBR green-based RT-qPCR. The gene expression data generated from several RT-qPCR experiments were integrated, analyzed by statistic software, and imported into CLADIST, a clustering tool associated with Protein, Signaling, Transcriptional Interactions and Inflammation Networks Gateway (pSTIING) (Ng et al., 2006), for heat map generation and hierarchical cluster analysis. The clustered genes with similar co-expression patterns were then cross-linked with biological pathways using BioCarta (http://biocarta.com/) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (Ogata et al., 1999) as general reference resources. Details of the statistic and bioinformatic analysis are described in Sections 2.5 and 2.6.

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