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Short Communication

A Short Note on TGF-β Signaling

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DESCRIPTION

Porcine Circo Virus-2 (PCV2) is recognized as an immunosuppressant. However, crosstalk between host cells in the signal transduction pathways associated with this virus remains poorly understood. In this study, the expression profiles of 84 genes involved in Transforming Growth Factor beta (TGF β) signaling were investigated in PCV2-infected porcine Primary Alveolar Macrophages (PAM) using the RT2 profiler PCR array system. Protein expression levels of cytokines involved in TGF-β signaling were determined using the Ray Biotech Fluorescent Pig Quantum Cytokine Array System [1]. Analyzed by a co-expression network, a number of genes involved in transcriptional regulation and apoptosis were differentially expressed in PCV2-infected PAM. Among these genes, TGF β, interleukin 10, CCAAT/enhancer-binding protein beta (C/EBPB), growth arrest and DNA damage inducing 45-beta (GADD45B) and BCL2 were up regulated. In contrast, SMAD family members 1 (SMAD1) and SMAD3 have been down regulated. These results suggested that PAM suppressed the TGF β signaling pathway at the onset of PCV2 infection. Suppression of apoptosis is indicated by up regulation of C/EBPB, GADD45B, BCL2, and down regulation of SMAD1 and SMAD3, prolonging the tolerance of PCV2 replication and potentially causing persistent infection. Porcine Circo Virus 2 (PCV2), a un-envelope, single-stranded circular DNA virus, is a major causative agent of PCV2 systemic disease (PCV2SD) and other PCV2 related diseases (PCVAD). PCVAD is the most economically important disease affecting the global pig industry. A notable feature of PCV2SD pigs is systemic immunosuppression [2]. Pigs with PCV2SD show severe depletion of lymphocytes in lymphoid tissue and marked reduction of T and B cells in peripheral blood, with lymphocyte depletion being the leading cause of immune suppression in PCV2 affected pigs. It suggests that there is a decreased lymphocyte proliferation and lysyl induced by PCV2, and apoptosis of primary lymphoid organs or progenitor cells are possible causes of lymphocyte depletion. Cytokines secreted by cells exposed to cellular stress are essential for the development of the immune response. Differential expression of several cytokines such as Interleukin (IL) 1/8/10/12 and TNF α has been reported in PCV2-infected porcine monocytes and macrophages. IL10 has been suggested to play an important role in PCV2 induced systemic immunosuppression [3]. Transforming Growth Factor beta (TGFβ) and its signaling pathways are contextually involved in cell proliferation, apoptosis, differentiation, migration and metastasis of various cell types. Deregulation of the TGF β signaling pathway is associated with many human diseases. In addition, it is a pleiotropic cytokine and has regulatory activity on several types of immune cells. T cells are considered to be important targets for TGF β and regulate T cell development, homeostasis, resistance and differentiation. TGF β plays a biological role primarily through standard SMAD signaling pathways. This pathway has three isoforms involved in multiple developmental processes such as TGFβ, TβR, and Smad.

Animals and Isolation of PAM Cells

The piglets were euthanized with an intravenous overdose of pentobarbital sodium prior to collecting the alveolar fluid. PAM has been isolated from the lungs in the previous way. Isolated PAM was adjusted to 1 x 10^7 cells/ml and cultured in growth medium containing RPMI 1640 supplemented with 10% (v/v) Fetal Bovine Serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin and incubated at 37°C, 5% CO_2.

Virus preparation for infection

A single layer of PK15 cells was inoculated at a density of 60%. After incubating at 37°C for 24 hours, the monolayer was treated with 200 mM D-glucosamine for 1 hour. After an additional 72 hours of incubation, cells were harvested and lysed 3 times by freeze-thaw. Genome copy of PCV2 in the lysed cell mixture was determined by pcr. PAM was infected with PCV2B with a Multiplicity of Infection (MOI) of 1 (1 viral DNA copy/cell). After infection, cells were cultured in RPMI1640 maintenance medium supplemented with 2% FBS and incubated at 37°C and 5% CO_2 for 1, 24, and 48 hours, respectively. Cells and supernatants were collected at the indicated time intervals, respectively. The cells were later stored in an RNA stabilizing reagent kit to prevent RNA degradation and stored at -70°C until use [4].

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Identification of PCV2 in PAMs

Post-infection PAM PCV2 was detected by Immune Fluorescence Assay (IFA). Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X100 in PBS. PCV2 in PAM was detected by a Quantum Dot binding specific single domain Antibody (QDspsdAb) probe.

RNA extraction and cDNA preparation

Total RNA was extracted using the RNA extraction kit as instructed. RNA content was measured with a micro spectrophotometer and RNA completeness was assessed by 1.8% agarose gel electrophoresis.

Analysis of TGF-β Expressions

The secreted TGF-β in the supernatant of PAMs post PCV2 infection was detected by Quant body porcine cytokine array system.

CONCLUSION

Monocyte or macrophage progeny cells play an important role in combating microbial infections. PCV2 has been shown to infect and persist cells of the immune system; including PAM. PCV2 alone can cause an extraordinary utilitarian weakness in PAM, reminiscent of poor phagocytosis and bactericidal activity. Recorded studies of PCV2 infected monocytes or macrophage stem cells have shown that stimulus and apoptosis-related properties are transmitted differently. In the current review, the target quality of the TGF-β signaling pathway in PCV2 infected PAM was investigated using the PCR exhibition framework. PCR array results showed that 58 of the 84 genes focused on the TGF-β signaling pathway were transmitted differently in PAM at various stages of post-PCV2 disease. These results suggest that the TGF-β signaling pathway may be involved in beneficial changes in PCV2 contaminated PAM. Curiously; the 48 grades were discriminatory controlled at 1hpi, achieving infection adsorption and disguise for the infection. Our results show that PAM showed a strong response to PCV2 between infection adsorption and veil. TGF β was totally up regulated at the mRNA and protein levels of PAM after PCV2 disease. In any case, SMAD1 and SMAD3 have been down regulated as the major mediators of the TGF-β signaling pathway of cell apoptosis. PCR cluster studies have shown that 58 of the downstream quality of the TGF-β signaling pathway are transmitted differently in PAM at various stages after PCV2 disease. Among these grades, IL-10 and TGF β are substantially up regulated, suggesting their cooperation in the pathogenesis of PCV2. Nonetheless, the suppression of PAM apoptosis exhibited by down regulated SMAD1 and SMAD3, and up regulated C/EBPB, GADD45B, BCL2 is part of the method of delaying PCV2 replication permission and is indomitable in clinical cases may be ill. It was triggered that a clear profile of TGF-β pathway-related qualities observed in PCV2 contaminated PAMs may represent practically variable elements in the invincible sequence of PCV2 affected pigs.

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