



Activation of the TLR/MyD88/NF- κ B Signal Pathway Contributes to Changes in IL-4 and IL-12 Production in Piglet Lymphocytes Infected with Porcine Circovirus Type 2 *In Vitro*

Dianning Duan, Shuxia Zhang, Xiaolin Li, Hua Guo, Mengmeng Chen, Yaqun Zhang, Junyuan Han, Yingjun Lv*

College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, China

Abstract

Porcine circovirus type 2 (PCV2) causes immunosuppression in pigs. One causative factor is an imbalance in cytokine levels in the blood and lymphoid tissues. Many studies have reported changes in cytokine production, but the regulatory mechanisms involved have not yet been elucidated. In this study, we investigated alteration and regulation of IL-4 and IL-12 production in lymphocytes following incubation with PCV2 *in vitro*. The levels of IL-4 decreased and levels of IL-12 increased in lymphocyte supernatants, and the DNA-binding activity of NF- κ B and the expression of p65 in the nucleus and p-I κ B in the cytoplasm of lymphocytes increased after incubation with PCV2. However, these effects were reversed when lymphocytes were coincubated with PCV2 and the NF- κ B inhibitor BAY11-7082. In addition, the expression of MyD88 protein increased and the expression of mRNA for the toll-like receptors (TLRs) TLR2, TLR3, TLR4 and TLR9 was upregulated when lymphocytes were incubated with PCV2. However, no change was seen in TLR7 and TLR8 mRNA expression. In conclusion, this study showed that PCV2 induced a decrease in IL-4 and an increase in IL-12 production in lymphocytes, and these changes were regulated by the TLR-MyD88-NF- κ B signal pathway.

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* E-mail: lyj@njau.edu.cn

Introduction

Porcine circovirus type 2 (PCV2) is a nonenveloped, single-stranded DNA virus with an unsegmented circular genome. It is the primary causative agent of postweaning multisystemic wasting syndrome (PMWS), which is characterized by generalized lymphadenopathy and tan-mottled, non-collapsed lungs [1]. PCV2 mainly affects weaning piglets, typically at 3–15 weeks, and has a morbidity rate of 5–15% [2–3]. In addition, PCV2 can cause immunosuppression in pigs, leading to secondary infection or coinfection with other pathogens, such as *Mycoplasma hyopneumoniae* and porcine reproductive and respiratory syndrome virus (PRRSV) [4–5].

Immunosuppression caused by PCV2 is mainly associated with lymphopenia and altered patterns of cytokine expression in blood and lymphoid tissues. The numbers of T and B lymphocytes and CD8⁺ and IgM⁺ cells have been reported to be markedly decreased in the blood, and lymphocyte depletion and histiocytic infiltration are found in lymphoid tissues of PMWS-affected pigs [1,6]. Lymphocyte depletion is associated with apoptosis and a reduction in lymphocyte proliferation, although the mechanisms are still unknown [7–10]. Altered patterns of cytokine expression are also found in piglets infected with PCV2. In pigs naturally

suffering from PMWS, IL-10 mRNA in the thymus and IFN- γ mRNA in the tonsils were overexpressed, while expression of IFN- γ , IL-10, IL-12p40, IL-4 and IL-2 mRNA was reduced in other lymphoid tissues [6]. In peripheral blood mononuclear cells harvested from pigs infected with PCV2, expression of IL-2, IL-6, IL-10, IL-12, TNF- γ and TNF- α mRNA was significantly increased and IL-4 mRNA expression slightly decreased compared with healthy pigs [11]. Although cytokine production has been shown to be disordered in pigs infected with PCV2, the underlying regulatory mechanisms remain largely unknown.

IL-4 and IL-12 belong to the Th2-type and Th1-type cytokine families and are associated with humoral and cellular immune responses, respectively. Altered patterns of IL-4 and IL-12 expression can reflect the immune status. Previous studies have suggested that monocyte and macrophage lineage cells and lymphocytes are the main target cells for PCV2 [12–13]. However, lymphocytes are the primary sites for PCV2 replication, while monocyte and macrophage cells are only the reservoir for PCV2 [13–14]. In addition, there are more lymphocytes in immune organs compared with monocyte and macrophage cells. Therefore it is necessary to investigate the altered cytokines induced by lymphocytes. In this study, changes in IL-4 and IL-12 expression and the regulatory mechanisms involved were investi-