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Short communication

Cytokine and chemokine mRNA expression profiles in tracheobronchial lymph nodes from pigs singularly infected or coinfected with porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (MHYO)

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ABSTRACT

The objective of this study was to determine cytokine and chemokine mRNA expression profiles in tracheobronchial lymph nodes from pigs singularly infected with porcine circovirus type 2 (PCV2), *Mycoplasma hyopneumoniae* (MHYO), or coinfected with both. Twenty-eight pigs were randomly assigned to one of four groups: (1) negative controls (NEG), (2) inoculated with MHYO (IMHYO), (3) inoculated with MHYO and PCV2 (CoI), and (4) inoculated with PCV2 (IPCV2). MHYO infection significantly ($P < 0.05$) stimulated innate cytokines, IL1B and IL8. PCV2 infection significantly stimulated expression of IFNG, IL8, NOS2A and chemokines CCL2, CCL5, and CXCL10. IFNB, IL1B and IL12 were slightly increased with PCV2 infection and IFNA and IL4 were significantly downregulated. Compared to NEG pigs, coinfection resulted in a significant increase in expression of IFNG, IL1B, IL8, CCL5, CXCL10, and weak stimulation of IFNB, IL6 and IL10; IL13 and IFNA were significantly downregulated. Overall MHYO potentiated PCV2 infection by increasing IFNG and IL10 mRNA expression levels. The increase of IFNG and chemokines and decrease of IFNA in IPCV2 and CoI pigs were correlated with increased severity of lymphoid lesions and the presence of PCV2 antigen. In summary, this work provided evidence that the increased severity of lesions in PCV2 and MHYO coinfected pigs was associated mainly with the presence of PCV2 antigen and alterations of cytokine mRNA expression profiles.

1. Introduction

Porcine circovirus (PCV)-associated disease (PCVAD) is increasingly recognized as a serious threat to the global swine production. The disease was first described as post-weaning multisystemic wasting syndrome (PMWS) (Allan et al., 1998). Today, PCV type 2 (PCV2) is generally recognized as the causative agent in the various clinical manifestations of PCVAD. In PCV2 infected pigs the distribution of the immune cell proportions within lymphoid tissues is altered, particularly with respect to lymphocyte populations (Allan and Ellis, 2000). Flow cytometric studies indicated depletion of specific blood lymphocyte populations of both field and experimentally infected pigs which appeared to be associated with the development of clinical disease (Nielsen et al., 2003; Segalés et al., 2001, 2005).

Mycoplasma hyopneumoniae (MHYO) is commonly identified in pigs suffering from the clinical presentation consistent with porcine respiratory disease complex (PRDC), originally described as porcine enzootic pneumo-

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nia, characterized by high morbidity but low mortality in affected herds (Goodwin et al., 1965; Maes et al., 1996). In the early stages of infection, MHYO typically colonizes the luminal surface of bronchial and bronchiolar epithelial cells as an extracellular pathogen (Amanfu et al., 1984; Kwon et al., 2002; Sarradell et al., 2003). Microscopically, the cilia are most affected, leading to a progressive loss, exfoliation of epithelial cells, and accumulation of inflammatory cells in and around the affected airway lumina (Blanchard et al., 1992). The hallmark lesion associated with MHYO is hyperplasia of the bronchus-associated lymphoid tissue (BALT) which may lead to airway obstruction of bronchioles and atelectasis of surrounding alveoli; but without MHYO antigen/nucleic acids (Kwon et al., 2002; Sarradell et al., 2003).

Under experimental conditions, pigs concurrently infected with MHYO and PCV2 had moderate dyspnea, lethargy, and reduced weight gain (Opriessnig et al., 2004). The overall severity of macroscopic lung lesions, PCV2-associated microscopic lesions in lung and lymphoid tissues, and the amount of PCV2-antigen associated with these lesions were significantly higher in coinfected pigs compared with singularly infected and control pigs; although PCV2 was enhanced by MHYO, a potentiating effect of PCV2 on MHYO replication and associated lesions was not observed (Opriessnig et al., 2004).

It has been determined that production of proinflammatory cytokines was associated with MHYO-induced pneumonia; increased levels of IL1, IL6, and TNFA were observed in broncho-alveolar lavage fluid (BALF) in MHYO infections (Asai et al., 1993, 1994; Thacker et al., 2000). Coinfection with MHYO and porcine reproductive and respiratory syndrome virus (PRRSV) significantly increased the severity and duration of pneumonia in experimentally infected pigs associated with induction of proinflammatory cytokines (Thacker et al., 2000). Changes in proinflammatory cytokine production may play a role in MHYO pathogenesis and persistence (Thanawongnuwech et al., 2004). Elevated levels of serum C-reactive protein (CRP) and IL10 were associated with PCV2 infected pigs that subsequently developed severe PMWS (Stevenson et al., 2006).

To further characterize the effect of MYHO on PCV2 the objective of this work was to determine if there were changes in mRNA expression of cytokines and chemokines in local lung-associated lymphoid tissues (tracheobronchial lymph nodes; TBLN) from pigs singularly infected or coinfected with PCV2 or MHYO.

2. Materials and methods

2.1. Animals

Twenty-eight, colostrum-fed, 2-week-old, crossbred, PCV2 seronegative specific-pathogen-free (SPF) piglets were purchased from a breeding herd with no history of PCVAD, free of MHYO and PRRSV. The pigs were housed in groups of seven pigs in four physically and environmentally separate rooms on raised wire decks of equal size and with identical thermal and ventilation controls. All animal manipulations were approved by the Iowa State University Institutional Animal Care and Use Committee (# 7-04-5704-S).

2.2. Experimental design and inoculation

The 28 pigs were randomly assigned to four groups of 7 pigs each: (1) negative controls (NEG), (2) inoculated with MHYO (IMHYO), (3) inoculated with MHYO and PCV2 (CoI), and (4) inoculated with PCV2 only (IPCV2). At five weeks of age, a tissue homogenate containing 10^5 color changing units/ml of MHYO strain 232 was administered intratracheally to each IMHYO and CoI pig (Opriessnig et al., 2004). For PCV2 inoculation PCV2 isolate ISU-40895, obtained through transfection of PK-15 cells, was used at $0.5 \times 10^{5.8}$ 50% tissue culture infective dose ($TCID_{50}$) per ml (Opriessnig et al., 2004). The sequence of infection (MHYO followed 2 weeks later by PCV2) was done similar to our previously published dual-infection model (Opriessnig et al., 2004) and based on our hypothesis that existing MHYO lesions are important for PCV2 replication. At seven weeks of age, CoI and IPCV2 pigs received 1 ml PCV2-40895 and 0.7 ml saline intramuscularly and 1 ml PCV2 and 0.8 ml saline intranasally (about $10^{4.7}$ $TCID_{50}$ per pig). A combination of intranasal and intramuscular route for PCV2 inoculation was done to ensure infection of each pig with a similar dose at the same time (Opriessnig et al., 2004). Each NEG and IMHYO pig was sham-inoculated at seven weeks of age with 1.8 ml PK15 cell supernatant intranasally and 1.7 ml intramuscularly.

2.3. Serology

Blood samples from individual pigs were collected on arrival, and at 0 and 14 days post PCV2 inoculation (DPI). Pigs were tested by a PCV2 open reading frame (ORF) 2 based ELISA and considered positive if the sample-to-positive (S:P) ratio was >0.2 (Nawagitgul et al., 2002). Serum samples taken on arrival and at necropsy were tested for the presence of antibodies to MHYO by an ELISA as previously described (Bereiter et al., 1990).

2.4. Clinical evaluation

After PCV2 challenge (2 weeks after MHYO), the pigs were monitored daily and scored for severity of clinical respiratory disease ranging from 0 (normal) to 6 (severe dyspnea and abdominal breathing) (Halbur et al., 1995) and evaluated daily for clinical signs including sneezing and coughing. Rectal temperatures and behavioral changes such as lethargy were recorded daily. Pigs were weighed on the day of PCV2 inoculation and at 7 and 14 DPI.

2.5. Necropsy, gross lesions and sample collection

At nine weeks of age, the pigs were humanly euthanized by an overdose of pentobarbital (Vortech Pharmaceuticals, Dearborn, MI, USA) and necropsied. Total macroscopic lung lesions were scored ranging from 0% to 100% of the lung surface being affected by consolidation and the size of lymph nodes ranging from 0 (normal) to 3 (4× the normal size) (Halbur et al., 1995; Opriessnig et al.,