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Cytokines Profile in Porcine Circovirus Type 2 Infection

[2]Wenkai Ren, [1,2]Liming Zeng, [2]Yinghui Li, [2]Li Wu, [2]Jieping Guo,
[2]Miaomiao Wu, [2]Gang Liu and [2]Yulong Yin
[1]College of Science, Jiangxi Agricultural University, 330045 Nanchang, China
[2]Key Laboratory of Agro-Ecological Processes in Subtropical Region,
Chinese Academy of Sciences, Scientific Observing and
Experimental Station of Animal Nutrition and Feed Science in South-Central,
Human Provincial Engineering Research Center of Healthy Livestock,
Institute of Subtropical Agriculture, Ministry of Agriculture, Changsha, 410125 Hunan, China

Abstract: Once a mammal infected by a microorganism an elaborate response will be generated that attempts to eliminate the infection agent. These responses contained innate immune responses and adaptive immune responses. Cytokines played a role in both innate and adaptive immune responses. Porcine circovirus type 2 was the causal agent of postweaning multisystemic wasting syndrome which was an economically important disease in pigs. Although, the interaction between immune responses and porcine circovirus was suggested as a determinant factor in the pathogenesis of postweaning multisystemic wasting syndrome, the reports about the cytokines profile in PCV2 infection were mixed and confused. Thus, a detailed review about this field was presented according to the literature and the lab's conclusion.

Key words: Cytokines, postweaning multisystemic wasting syndrome, porcine circovirus, pigs, profile

INTRODUCTION

Complex immune responses in mammal were generated after infected with bacterium or virus. These immune responses were grouped into two families, innate immune responses (un-specific responses) and adaptive immune responses (specific responses). These immune responses played a role in controlling infection by pathogens and consisted of a large number of elements. One of the most important components of the immune responses was cytokines. Porcine Circovirus type 2 (PCV2) was presumed as the essential pathogen for the Post-weaning Multisystemic Wasting Syndrome (PMWS) (Allan *et al*., 1998; Ellis *et al*., 1998) which had been reported worldwide and caused a big economical loss in pig production.

As an immunosuppressive disease, the interaction between PCV2 and the immune system was suggested as a determinant factor in the pathogenesis of PMWS (Darwich *et al*., 2003a; Fort *et al*., 2009). To gain insights into the host immune mechanism developed against PCV2 infection, many researches were proceeded to approach immune responses on PCV2 infection. In these studies, the most parameters measured were cytokines and chemokines (at less degree) because their role in diverse pathologies, cell growth and differentiation, immune regulation, inflammatory response and wound healing (Fort *et al*., 2009). However, the results about the effect of PCV2 infection on cytokines profile were mixed and confused because of the measurement time, measurement position, measurement methods and animal models. Here, researchers reviewed the cytokines profile in PCV2 infection according to the literatures and the lab's results. The primary purpose is to facilitate the researchers' work by providing the general cytokines profile with PCV2 infection to them. The most cytokines were studied in PCV2 infection were interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-8, interleukin-10, interleukin-12, alpha interferon, gamma interferon and tumor necrosis factor alpha.

INTERLEUKIN-1

Interleukin-1 (IL-1), secreted by many kinds of cells (such as monocytes, macrophages) was a family of proteins containing IL-1α, IL-1β, IL-1Rα and IL-18

Corresponding Author: Yulong Yin, Key Laboratory of Agro-Ecological Processes in Subtropical Region, Chinese Academy of Sciences, Scientific Observing and Experimental Station of Animal Nutrition and Feed Science in South-Central, Human Provincial Engineering Research Center of Healthy Livestock, Institute of Subtropical Agriculture, Ministry of Agriculture, Changsha, 410125 Hunan, China

Table 1: The IL-1 profile in different experimental

References	Experimental model	Measure time	Measure methods	Measure position	Profile
Eckmann *et al.* (1993)	Women with endometriosis	Not mention	ELISA	Serum and peritoneal fluid	Increase
Ellis *et al.* (1998)	Rabbit with incised wound	**	Real-time fluorescent, quantitative PCR	Skin and postmortem	*
Farrar and Schreiber (1993)	Women with ovarian cancer	No exact time	ELISA	Serum, ascites or peritoneal	⊠
Flexner *et al.* (1987)	BABL/c mice infected with JEV	2 and 4 DPI RT-PCR for mRNA levels	☆	Brain	Western blot for protein levels
Fort *et al.* (2009)	PBMP infected with HIV-1	Few hours after exposured to virus	★	Surpernant	Increase

**: <0.5, 0.5, 1, 2, 3, 4,5, 6, 8, 12 and 24 h, 2, 3 and 7 days for skin; 1, 2 and 6 h for postmortem; *: IL-1β mRNA in rabbit skin significant increased at <0.5 h, reached the peak level at 2 h after incised wounds, decreased and was almost normalized at 2 days, however, no significant increase for IL-1βmRNA in postmortem samples; ⊠: IL-1β increased in ascites or peritoneal fluid and IL-1 RA increased in serum, ascites or peritoneal; ☆: the mRNA expression of IL-18 wasn't changed at 2 days post infection but enhanced by 2.5 folds at 4 days post infection; IL-1β also showed 2 fold and 2.2 fold increase in expression on the 2 and 4 days post infection, respectively. The protein levels of IL-18 increased to 2 folds and IL-1β increased to 2.5 folds at 4 days post infection; ★: couldn't to validate for lack of literature ; JEV: Japanese Encephalitis Virus. ELISA: Enzyme Linked Immunosorbent Assay, PBMP: Peripheral Blood Mononuclear Phagocytes, HIV-1: Human Immunoddficiency Virus type I, DPI: Days Post Infection, IL-1: Interleukin-1, HIV-1: Human Immunodeficiency Virus type 1

(March *et al.*, 1985). IL-1 was one of the pivotal early response pro-inflammatory cytokines that through up or down regulation of other cytokines, enabled organisms to respond to infectious non-self challenges and induced a cascade of effects leading to inflammation (Dinarello, 1997). Gene expression of IL-1 could be induced very sharply by many stimuli such as activators of inflammation (bacterial products, other cytokines), anti-inflammatory molecules (Dinarello, 1998). Many evidences could indicate that IL-1 increased in the inflammation (Table 1). For example, Anasz found that the serum and peritoneal fluid IL-1α and IL-1Rα levels in the women with endometriosis were higher than the normal control (Kondera-Anasz *et al.*, 2005). Bai *et al.* (2008) found that the expression of IL-1β mRNA in rabbit skin significant increased at <0.5 h, reached the peak level at 2 h after incised wounds, decreased and was almost normalized at 2 days. Meanwhile, Mustea *et al.* (2008) also found that the concentrations of IL-1β in ascites or peritoneal fluid and IL-1 RA in serum, ascites or peritoneal were significantly increased in patients with ovarian cancer in comparison to the control group. This phenomenon also been found in virus infection such as Das *et al.* (2008) found that the IL-18 and IL-1β in BALA/c mice brain increased in both mRNA levels and protein levels with progressive Japanese encephalitis virus infection. Merrill *et al.* (1989) also found that Human Immunoddficiency Virus (HIV) could induce IL-1 secretion. However, the IL-1 profile in PCV2 infection was mixed. Darwich *et al.* (2003a, b) reported that no difference was found about IL-1 β expression level in inguinal and bronchial lymph nodes, tonsils, spleen and thymus of the pigs infected with PCV2. In agreement with this result, An *et al.* (2008) reported that no difference about IL-1α and IL-1β levels were found in mice after vaccinated with naked DNAs encoding six open reading frame antigens of PCV2. Fort *et al.* (2009) reported that the serum IL-1β level, most piglets were negative and it was only detected sporadically regardless of the PCV2 inoculation status. Sipos *et al.* (2005) reported that no notable differences in IL-1β protein expressions be found. Moreover, Chang *et al.* (2006) also tested that the IL-1β levels produced were equally low in PCV2 and mock-inoculated. Differently, reports indicated that IL-1 was increased with PCV2 infection were existed. Sipos *et al.* (2004) found that IL-1α mRNA levels and intracellular IL-1β levels were notably increased in the pigs suffering from the natural PMWS and IL-1α mRNA level significantly elevated in PDNS pigs (Sipos *et al.*, 2005). Chae and Choi (2011) reported that the mRNA expressions of IL-1α was significantly up-regulated in pigs with PCV2-associated respiratory disease. Zhang *et al.* (2011) also found that the express of IL-1β was slightly and significantly increased with PCV2 infection, PCV2 and *Mycoplasma hyopneumoniae* coinfection, respectively.

INTERLEUKIN-2

Interleukin 2 (IL-2) was one of the first cytokines to be discovered and cloned (Taniguchi *et al.*, 1983). It secreted by activated T lymphocytes, especially by activated $CD4^+$ T-helper cells and $CD8^+$ T-helper cells (Keene and Forman, 1982) and named T-Cell Growth Factor (TCGF) for it stimulated T cells proliferation and differentiation (Morgan *et al.*, 1976; Smith, 1988). IL-2 could promote the activity of immune cells to kill cancer cells, abnormal cells infected by virus and bacteria (Flexner *et al.*, 1987; Allen *et al.*, 1990). Accordingly, IL-2 is pivotal for the generation and regulation of acquired immune responses. About IL-2 in PCV2 infection, most reports indicated that PCV2 infection could down-regulate the IL-2 mRNA expression.

For example, Darwich *et al.* (2003a, b) found IL-2 expression level decreased in spleen and inguinal lymph nodes of the pigs infected with PCV2. Sipos *et al.* (2004) found that IL-2 mRNA level trend to be down-regulated in the pigs suffering from the natural PMWS, PDNS and PMWS pigs. Furthermore, co-stimulation of PRV with either PCV1 or PCV2 reduced IL-2 production from Peripheral Blood Mononuclear Cells (PBMCs) on average by 50 and 80%, respectively (Kekarainen *et al.*, 2008). Interestingly, IL-2 protein level was increased in many reports even one found that IL-2 mRNA level decreased but IL-2 protein level increased at the same time (Sipos *et al.*, 2004, 2005). However, existed reports also indicated that PCV2 infection had no effect on IL-2 production. Such as Darwich *et al.* (2003a, b) reported that no difference was found about IL-2 expression level in bronchial lymph nodes, tonsils and thymus. An *et al.* (2008) reported that no difference about IL-2 level was found in mice after vaccinated with naked DNAs encoding six open reading frame antigens of PCV2. Fort *et al.* (2009) reported that after *in vitro* treatment of PBMC with PCV2, no induction on IL-2 was observed. Zhang *et al.* (2011) found that the express of IL-2 in control group was similar with PCV2 infection group, PCV2 and *Mycoplasma hyopneumoniae* co-infection group. Although, Shi *et al.* (2010) found that IL-2 mRNA level had significantly reduced in Porcine Respiratory and Reproductive Syndrome Virus (PRRSV)/PCV2 co-infected piglets compared to those of the piglets infected with either PRRSV or PCV2 alone, no difference was found between the PCV2 infected and the control.

INTERLEUKIN-4

Interleukin-4 (IL-4) was a pleiotropic cytokine generally associated with cellular activation, differentiation and rescue from apoptosis. It was secreted by activated T lymphocytes, mast cells and basophils (Hou *et al.*, 1994). As IL-4 stimulated acivity of B cells and proliferation of activated B cells also enhanced antigen presenting capacity of B cell, it was called B cell stimulating factor. Besides, it played an important role in immunoglobulin class switching and T cell polarization to the Th2 phenotype. Moreover, IL-4 induced increased DNA binding activity of transcription factors that was important for collagen synthesis so that it activated intermediate proteins involved in the growth and proliferation of fibroblasts (McGaha *et al.*, 2003).

Most reports indicated IL-4 was down-regulated in PCV2 infection. Darwich *et al.* (2003a, b) found IL-4 expression level decreased in tonsils and inguinal lymph nodes of the pigs infected with PCV2. Sipos *et al.* (2005) showed that IL-4 mRNA level was notably decreased in both PDNS and PMWS pigs, IL-4 protein level in PMWS pigs lower than the group (Sipos *et al.*, 2004, 2005. Shi *et al.* (2010) found that IL-4 mRNA level had significant reduced in PRRSV/PCV2 co-infected piglets at 7, 14, 21 and 42 days post-infection, in PCV2 piglets at 7 days post-infection. Zhang *et al.* (2011) also found that the express of IL-4 mRNA were significant down-regulated in PCV2 infection. However, some papers reported a different result. Sipos *et al.* (2004) found that no difference about IL-4 mRNA level was found in the pigs suffering from the natural PMWS. An *et al.* (2008) reported that no difference about IL-4 level was found in mice after vaccinated with naked DNAs encoding six open reading frame antigens of PCV2. In agreement with that Fort *et al.* (2009) also, reported that after *in vitro* treatment of PBMC with PCV2, no induction on IL-4 was observed. Even Sipos *et al.* (2005) reported that the IL-4 protein level in PDNS pigs was higher than the control group.

INTERLEUKIN-6

Interleukin 6 (IL-6) was a multifunctional cytokine synthesized by various cells including activated T and B cells, mononuclear macrophages, endothelial cells, epithelial cells and fibroblasts. IL-6 played a very complex role in biological events including immune responses, hematopoiesis and regulation of the endocrine and nervous systems (Biffl *et al.*, 1996; Naugler and Karin, 2008). Unlike IL-4, IL-6 was upregulated in most PCV2 infection. A slight increase was observed for IL-6 mRNA expression and protein level in the pigs suffering from the natural PMWS (Sipos *et al.*, 2004) and IL-6 mRNA expression and IL-6 protein level were increased in PDNS pigs (Sipos *et al.*, 2005). Shi *et al.* (2010) also found that IL-6 mRNA level had significant increased in PCV2 infected piglets at 7, 14, 21, 28 and 42 Days Post-Infection (DPI) in PCV2/PRRSV co-infection piglets at 7 and 42 days post-infection. An *et al.* (2008) reported that ORF5 resulted in high IL-6 expression on DPI 35 in mice after vaccinated with naked DNAs encoding six open reading frame antigens of PCV2. Disagreement with above, no significant difference was found in PMWS pigs (Sipos *et al.*, 2005). In agreement with this, Zhang *et al.* (2011) also, found that level of IL6 was not different in the PCV2 infection groups compared to negative pigs. Moreover, Stevenson *et al.* (2006) also found that no significant differences in IL-6 expression in the PMWS-affected piglets compared to the subclinically infected piglets.

INTERLEUKIN-8

Interleukin-8 (IL-8) was a chemokine produced by monocytes (Baggiolini and Clark-Lewis, 1992; Grau *et al.*, 2001) and other cell types such as epithelial cells and

endothelial cells (Utgaard *et al*., 1998; Wolff *et al*., 1998), cytokine activated human vascular smooth muscle cells (Wang *et al*., 1991). It was often associated with inflammation by acting preferentially on neutrophils (Baggiolini *et al*., 1995). Expression of IL-8 could be regulated by inflammatory cytokines such as IL-1β and tumor necrosis factor-alpha (Akiba *et al*., 2001). Its production could be induced by many other factors such as tumor, bacteria, virus and even iron present in coal fly ash and trigger a inflammation response (Eckmann *et al*., 1993; Bruder and Kovesdi, 1997; Waugh and Wilson, 2008). Researchers could found that IL-8 expression and protein level increased in PCV2 infection. A slight increase of expression was observed for IL-8 in the pigs suffering from the natural PMWS (Sipos *et al*., 2004). IL-8 production in PCV2-inoculated Alveolar Macrophages (AMs) was also persistently up-regulated about two to five fold the corresponding mock-inoculated AMs (Chang *et al*., 2006). Fort *et al*. (2009) reported that all PCV2-inoculated piglets had a transient increase in plasma levels of IL-8 (184.8±37.5 pg mL^{-1}) by 1DPI whereas uninoculated controls remained negative. The mRNA expressions of IL-8 was significantly up-regulated in pigs with PCV2-associated respiratory disease (Chae and Choi 2011). Few reports showed a different results such as Darwich *et al*. (2003a, b) reported that no difference was found about IL-8 expression level in inguinal and bronchial lymph nodes, tonsils, spleen and thymus of the pigs infected with PCV2. Zhang *et al*. (2011) found that the express of IL-8 was significantly decreased with PCV2 infection, PCV2 and *Mycoplasma hyopneumoniae* co-infection.

INTERLEUKIN-10

IL-10 was a multifunctional cytokine synthesized by cells including T, B lymphocytes, macrophages and certain subsets of dendritic cells (Wilson *et al*., 2005). Its prime function was to inhibit many functions of NK cells, T cells and macrophage and dendritic cells, reduce production of inflammatory cytokines (Trinchieri, 2007; O'Garra *et al*., 2008). Due to its anti-inflammatory property, IL-10 had been widely used in some chronic inflammatory diseases such as asthma and autoimmune diseases (Zhou *et al*., 2010).

Large number of evidences indicated IL-10 expression increased in PCV2 infection. Elevated IL-10 level was consistently detected in the PMWS-affected piglets from 10DPI and was significantly higher by 14 DPI. Compared to both the subclinically infected and control piglets (Stevenson *et al*., 2006). IL-10 was notably increased in the nature PMWS affected animals (Sipos *et al*., 2004).

IL-10 production from PBMCs was elevated with PCV2 stimulation or PCV2/Pseudorabies Virus (PRV) co-stimulation (Kekarainen *et al*., 2008). The level of IL-10 detected in supernatants of PBMC stimulated with PCV2 was significantly higher than those detected in mock-stimulated cultures (Fort *et al*., 2009). Shi *et al*. (2010) found that IL-10 mRNA level had significant increased in PCV2 infected and PCV2/PRRSV co-infection piglets at 7, 14, 21, 28 and 42 days post-infection. An *et al*. (2008) reported that ORF2 resulted in high IL-10 expression on DPI 35 in mice after vaccinated with naked DNAs encoding six open reading frame antigens of PCV2. Inoculated animals with PCV2 produced higher levels of IL-10 than the controls at week 3 PI and correlation between viral load and IL-10 amounts was observed (Darwich *et al*., 2008). However, different results were existed and these results were confused. Sipos *et al*. (2005) found that the expression of IL-10 in the PMWS group was significantly ($p<0.05$) decreased compared to control pigs. Contrarily, Zhang *et al*. (2011) found that the express of IL-10 was no difference with PCV2 infection, PCV2 and *Mycoplasma hyopneumoniae* co-infection. More confused, Darwich *et al*. (2003a, b) reported that no difference about IL-10 expression level in bronchial lymph nodes, tonsils and spleen, increased IL-10 expression level in thymus; decreased IL-10 expression level in inguinal lymph nodes were found in the pigs infected with PCV2. Chae and Choi (2011) reported that IL-10 mRNA expression was not detected in the lungs of pigs with PCV2-associated respiratory disease.

INTERLEUKIN-12

Interleukin-12 (IL-12), synthesized primarily by monocytes and macrophagocytes was a heterodimeric cytokine composed of two disulfide-linked subunits, 35 and 40 kDa, respectively designated as p35 and p40. When co-expression in the same cell, these subunits formed the bioactive p70 heterodimer whose main function was to promote cytotoxic functions of T and NK cells (Watford *et al*., 2003). Most reports showed that PCV2 infection inhibited the IL-12 expression. For example, Darwich *et al*. (2003a, b) found that decreased IL-12 expression level in spleen and inguinal lymph nodes were found in the pigs infected with PCV2. An *et al*. (2008) reported that IL-12 level was decreased on DPI 7 in mice after vaccinated with naked DNAs encoding 2, 3 and 4 open reading frame antigens of PCV2 (An *et al*., 2008). Kekarainen *et al*. (2008) also shown that PCV2 and to a lesser extent PCV1 may inhibit IL-12 secretion upon a recall stimulation of PBMCs. Zhang *et al*. (2011) also found that the express of IL-12B was decreased with PCV2

Table 2: The IFN-γ profile in different experimental

References	Experimental model	Measure time	Measure methods	Measure position	Profile
Thyrell *et al.* (2002)	Chicken with REV or REV+CAV	**	Quantitative real-time PCR and RT-PCR	Embryo fibroblast	Increase
Trinchieri (2007)	HIV-infected patients with IL-2 treated	No exact time	ELISA	PBMCs	Increase
Utgaard *et al.* (1998)	Ducks with hepatitis B virus	3-14DPI	Quantitative RT-PCR	Liver	Increase

**: 2 weeks old SPF chicks or 10 days old chicken embryos; ELISA: Enzyme Linked Immunosorbent Assay, IFN-γ: Interferon Gamma, IL-2: Interleukin-2; PBMCs: Peripheral Blood Mononuclear Cells, DPI: Days Post Infection, HIV: Human Immunoddficiency Virus, REV: Reticuloendotheliosis Virus, CAV: Chicken Anemia Virus

infection. As same as other cytokines, different results were existed. Darwich *et al.* (2003a, b) reported that no difference about IL-12 expression level in bronchial lymph nodes, tonsils and thymus in the pigs infected with PCV2. Sipos *et al.* (2004) reported that IL-12 mRNA level was found no difference in PMWS pig and PDNS pig (Sipos *et al.*, 2005) but IL-12 protein level was highly significantly elevated in PDNS pig in comparison to controls (Sipos *et al.*, 2005). Even Shi *et al.* (2010) found that IL-12p40 mRNA level had significant increased in PCV2 infected and PCV2/PRRSV co-infection piglets.

GAMMA INTERFERON

Interferon gamma (IFN-γ) was a multifunctional protein first observed as an antiviral activity in cultures of Sindbis virus-infected human leukocytes stimulated by PHA. Later research indicated that it induced anti-viral, anti-proliferative and immunomodulatory effects on target cells (Farrar and Schreiber, 1993). IFN-γ had historically been considered as a product solely of T cells and NK cells however, it was discovered recently that with appropriate stimulation, B cells, macrophages and Dendric Cell (DC) could produce IFN-γ (Puddu *et al.*, 1997; Yoshimoto *et al.*, 1997; Ohteki *et al.*, 1999). Usually, INF-γ level was upregulated after virus infection (Table 2). Schat demonstrated INF-γ transcript increased about 10 fold in INF-γ mRNA levels at 7 DPI following Reticuloendotheliosis Virus (REV) or REV/Chicken Anemia Virus (CAV) co-infection and two to four fold increased in CAV infection (Markowski-Grimsrud and Schat, 2003). Sabbatini *et al.* (2010) also found that INF-γ production increased in IL-2 treated HIV-infected patients. Furthermore, Narayan *et al.* (2006) found that duck INF-γ (DuIFN-γ) RNA level was up-regulated rapidly from day 3 after infection in the liver samples from hepatitis B virus infected duck.

The results in this field were mixed. Some reports showed that IFN-γ expression increased after infection. Shi *et al.* (2010) found that IFN-γ mRNA level had significant increased in PCV2 infected and PCV2/PRRSV co-infection piglets. Sipos *et al.* (2005) also showed that IFN-γ mRNA expressions and protein level were found to be elevated in PDNS pigs. An *et al.* (2008) reported that vaccination with ORF1-encoding naked DNA elevated IFN-γ level. Zhang *et al.* (2011) found that the express of IFN-γ was increased with PCV2 infection, PCV2 and *Mycoplasma hyopneumoniae* co-infection. Disagreement with above, Kekarainen *et al.* (2008) showed that PCV2 and to a lesser extent PCV1, inhibited IFN-γ secretion upon a recall stimulation of PBMCs. Darwich *et al.* (2003a, b) reported that no difference about IFN-γ expression level in bronchial lymph nodes, spleen and thymus and decreased IFN-γ expression in inguinal lymph nodes were found in the pigs infected with PCV2. Sipos *et al.* (2004) also indicated an interesting result that IFN-γ mRNA expression appeared to be slightly increased but intracellular level was slightly decreased in the diseased animals. Even had research tested that IFN-γ wasn't expressed with PCV2 infection such as Fort *et al.* (2009) reported that no significantly induction on IFN-γ was observed after *in vitro* treatment of PBMC with PCV2 and mRNA was expressed in only two of ten animals of pigs with PCV2-associated respiratory disease and its expression was very weak (Chae and Choi, 2011).

ALPHA INTERFERON

IFN-α was a member of the type I interferon family which was active as an antiviral and immuno-modulatory cytokine (Cella *et al.*, 1999; Siegal *et al.*, 1999). In humans, the production of IFN-α was most efficiently induced in many types of immune cells upon viral infection (Taniguchi and Takaoka, 2001). IFN-α had been used clinically in the therapy of some malignancies and viral diseases. Several studies have shown that IFN-α induced a strong and direct apoptotic response in primary malignant cells and in tumor cell lines *in vitro* (Sangfelt *et al.*, 1997; Otsuki *et al.*, 1998; Thyrell *et al.*, 2002). Many reports indicated that IFN-α level was increased in inflammation and virus infection (Table 3). However, IFN-α production was inhibited with PCV2 infection (Vincent *et al.*, 2007; Kekarainen *et al.*, 2008). Zhang *et al.* (2011) also showed that IFN-α was decreased in PCV2 infection and PCV2/*Mycoplasma hyopneumoniae* co-infection pigs. Meanwhile, there were no significant differences in IFN-α expression between the PMWS-affected piglets and the subclinically infected piglets (Stevenson *et al.*, 2006) and it could be measured in serum by day 5 PI, later on, positive results were only sporadically detected (Fort *et al.*, 2009).

Table 3: The IFN-α profile in different experimental

References	Experimental model	Measure methods	Measure position	Profile
This study	Influenza A and Sendai virus-infected human	Flow cytometry and Western blot	Myeloid dendritic cells	Increase
This study	Patients with systemic lupus erythematosus	Quantitative real-time RT-PCR	Human lupus	Increase
This study	HIV-1-infected patients	Immunofluorescence microscopy	Tonsil	Increase

IFN-α: Interferon alpha; HIV: Human Immunoddficiency Virus

TUMOR NECROSIS FACTOR-ALPHA

Tummour Necrosis Factor-alpha (TNF-α), secreted as a 26 kDa transmembrane monomer (mTNF-α) by both macrophages and monocytes was an important member of TNF family (Cawthorn and Sethi, 2008). TNF family had been expanded to 19 members including the most concerned TNF-α and lta since, their first molecular cloning of cDNAs in the early 1980s (Locksley *et al*., 2001; Bodmer *et al*., 2002). The TNF family proteins were expressed as transmembrane type-II proteins with the exception of LT-α (Wallach *et al*., 1997). It had a key role in immune regulation, increasing lymghoid development, cell proliferation, differentiation, activation and death (Smyth and Johnstone, 2000; Ch'en *et al*., 2005). Now a days, TNFα had been of intensitve interests with diverse functions, mainly its cytolytic and cytostatic ability to a wide range of cancer cells without harming normal cells. Niu *et al*. (2009) observed significant up-regulation of TNFα mRNA at 30 min of coronary artery occlusion in rats. Kaoutar proved that HIV-1 Tat protein was able to induce TNF-α production in human macrophages (Leghmari *et al*., 2008).

Most researches showed that TNF-α was increased with PCV2 infection. TNF-α production in alveolar macrophages with experimentally infected with PCV2 and combination of PCV2 and PPV was higher than in control group (Kim *et al*., 2006). In PCV2-inoculated AMs, the level of TNF-α was significantly increased (Chang *et al*., 2006). Shi *et al*. (2010) also found that TNF-α mRNA level had significant increased in PCV2 infected and PCV2/PRRSV co-infection piglets. Moreover, this idea could be tested by Sipos *et al*. (2004) who found that TNF-α mRNA expression appeared to be increased in the pigs infected with PCV2 and intracellular TNF-α level expression was significant increased in PDNS pigs (Sipos *et al*., 2005). Further research indicated that ORF3 elicited high levels of the pro-inflammatory cytokine TNF-α level (An *et al*., 2008). Unfortunately, Fort *et al*. (2009) reported that this cytokine was only detected sporadically regardless of the PCV2 inoculation status.

CHEMOKINES

Chemokines were small proteins with 70-80 amino acids in size. It could be conveniently grouped into two categories, housekeeping chemokines and proinflammatory chemokines which recruited immune cells to sites of infection, inflammation and tissue damage. Chemokines also could be divided into four classes, CXC, CC, C and CX3C, on the basis of differences in the position of cysteines within a conserved four-cysteine motif. Alvelolar Macrophage-derived neutrophil Chemotactic Factor-II (AMCF-II), produced by alvelolar macrophage and belonged to the CXC subfamily (Smith *et al*., 1997). This chemokine was specific neutrophils chemoattractant and could recruit neutrophils to the lesion area (Goodman *et al*., 1992; Burdon *et al*., 2008). Macrophage Chemoattractant Protein (MCP) and Macrophage Inflammatory Protein-1(MIP-1) belongs to the CC subfamily were a powerful chemoattractant for monocytes and macrophages (Wolpe *et al*., 1988; Oppenheim *et al*., 1991; Rollins 1997). Maximum MCP-1 expression and MIP-1 expression in lymph nodes were observed at 17 and 21 days post-inoculation (dpi) of intranasally with PCV2, respectively (Kim and Chae, 2004). The relative mRNA levels of porcine AMCFII, MCP-1, CCL5 and MCP-2 were all strongly up-regulated following PCV2 inoculation Ams (Chang *et al*., 2006). Zhang *et al*. (2011) also showed that CCL2,CCL5 and CXCL10 were increased in PCV2 infection and PCV2/*Mycoplasma hyopneumoniae* co-infection pigs (Table 4).

Table 4: The chemokines profile with PCV2 infection

Catalogue	Profile	References
MCP-1	↑	Keene and Forman (1982)
MIP-1	↑	This study
AMCFII	↑	Keene and Forman (1982)
MCP-2	↑	Keene and Forman (1982)
CCL2	↑	Kim and Chae (2004)
CCL5	↑	Keene and Forman (1982) and Kim and Chae (2004)
CXCL10	↑	Kim and Chae (2004)

↑: Increase; MCP-1: Monocyte Chemotactic Protein-1; MIP-1: Macrophage Inflammatory Protein-1; AMCF-II: Alvelolar Macrophage-derived neutrophil Chemotactic Factor-II; MCP-2: Macrophage Chemoattractant Protein 2; CCL2: Monocyte Chemotactic protein-1/monocyte chemotactic and activating factor; CCL-5: Regulated upon activation, normal T cell expressed and secreted factor; CXCL10: interferon r-induced protein-10

DISCUSSION

From the literature reports (Table 5) and the detected results in the lab of these years, researchers can make a conclusion that the IL-1, IL-6, IL-8, IL-10, TNF-α, IFN-γ and chemokines levels were increased with PCV2 infection but IL-2, IL-4, IL-12 and IFN-γ levels were decreased.

Table 5: The cytokines profiles in PCV2 infection

	IL-1				
References	Experimental model	Measure time	Measure methods	Measure point	Profile
Kekarainen *et al.* (2008)	Pig	Not mentioned in mRNA level Short-time after stimulating in protein level	Semiquantitative PT-PCR for mRNA levels FCICD for protein level	Blood Intracellular of PBMCs	#
Hou *et al.* (1994)	Pig	Not mentioned in mRNA level Short-time after stimulating in protein level	Semiquantitative PT-PCR for mRNA levels FCICD for protein level	Blood Intracellular of PBMCs	@
Keene and Forman (1982)	Swine AMs	18-108HPI	ELISA	Culture supernatants	No difference
Grau *et al.* (2001)	Mice	7 and 35 DPI with naked DNAs	Bio-Plex cytokines assays	Blood	No difference
Allen *et al.* (1990)	Pig	0, 7, 14, 21 and 29DPI	Capture ELISAs	Serum	Couldn't be detected
Kennedy *et al.* (2000)	Pig	No mentioned	RT-PCR	Lung	Increase
Kim and Chae (2004)	Pig	14 and 28 DPI	RT-PCR	Tracheobronchial IL-1a lymph nod IL-1β increase	No difference
Goodman *et al.* (1992)	Pig	1 week suffering a progression and respiratory disorders	RT-PCR	Inguinal, bronchial lymph nodes, tonsils, spleen and thymus	No difference

	IL-2				
References	Experimental model	Measure time	Measure methods	Measure point	Profile
Goodman *et al.* (1992)	Pig	1 week suffering a progression and respiratory disorders	RT-PCR	Inguinal, bronchial lymph nodes, tonsils, spleen and thymus	**
Kekarainen *et al.* (2008)	Pig	Not mentioned in mRNA level short-time after stimulating in protein level	Semi-quantitative PT-PCR for mRNA levels FCICD for protein level	Blood Intracellular of PBMCs	*
Hou *et al.* (1994)	Pig	Not mentioned in mRNA level short-time after stimulating in protein level	Semi-quantitative PT-PCR for mRNA levels FCICD for protein level	Blood Intracellular of PBMCs	*
McGaha *et al.* (2003)	PBMCs from PRV-immunized pigs	Not mentioned	ELISA	Culture supernatants	Decrease
Grau *et al.* (2001)	Mice	7 and 35 DPI with naked DNAs	Bio-Plex cytokines assays	Blood	No difference
Allen *et al.* (1990)	PBMCs from PCV2 infection	Not mentioned	Capture ELISAs	Cell culture supernatants	No difference
Meerts *et al.* (2005)	PBMCs from PCV2/PRRSV or PCV2 infected piglets	0, 7, 14, 21, 28 and 42 DPI	Quantitative RT-PCR	PBMCs	⊠
Kim and Chae (2004)	Pig	14 and 28 DPI	RT-PCR	Tracheobronchial lymph difference nodes	No

	IL-4				
References	Experimental model	Measure time	Measure methods	Measure point	Profile
Goodman *et al.* (1992)	Pig	1 week suffering a progression and respiratory disorders	RT-PCR	Inguinal, bronchial lymph nodes, tonsils, spleen and thymus	§
Kekarainen *et al.* (2008)	Pig	Not mentioned in mRNA level short-time after stimulating in protein level	Semiquantitative PT-PCR for mRNA levels FCICD for protein level	Blood Intracellular of PBMCs	″
Hou *et al.* (1994)	Pig	Not mentioned in mRNA level Short-time after stimulating in protein level	Semiquantitative PT-PCR for mRNA levels FCICD for protein level	Blood Intracellular of PBMCs	№
Grau *et al.* (2001)	Mice	7 and 35 DPI with naked DNAs	Bio-Plex cytokines assays	Blood	Nodifference
Allen *et al.* (1990)	PBMCs from PCV2 infection	Not mentioned	Capture ELISAs	Cell culture supernatants	Nodifference
Meerts *et al.* (2005)	PBMCs from PCV2/PRRSV or PCV2 infected piglets	0, 7, 14, 21, 28 and 42 DPI	Quantitative RT-PCR	PBMCs	○
Kim and Chae (2004)	Pig	14 and 28 DPI	RT-PCR	Tracheobronchial lymph nodes	Decrease

Table 5: Continue

IL-6

References	Experimental model	Measure time	Measure methods	Measure point	Profile
Kekarainen *et al.* (2008)	Pig	Not mentioned in mRNA level Short-time after stimulating in protein level	Semiquantitative PT-PCR for mRNA levels FCICD for protein level	Blood Intracellular of PBMCs	Increase
Hou *et al.* (1994)	Pig	Not mentioned in mRNA level	Semiquantitative PT-PCR for mRNA levels	Blood	In crease in PDNS pigs
		Short-time after stimulating in protein level	FCICD for protein level	Intracellular of PBMCs	No difference in PMWS pigs
Naugler and Karin (2008)	Pig	0, 3, 7, 10, 14, 21, 28 and 35 DPI	Bioassay	Serum	No difference
Grau *et al.* (2001)	Mice	7 and 35 DPI with naked DNAs	Bio-Plex cytokines assays	Blood	increased with ORF5 vaccination on DPI 35
Meerts *et al.* (2005)	PBMCs from PCV2/PRRSV or PCV2 infected piglets	0, 7, 14, 21, 28 and 42 DPI	Quantitative	RT-PCR PBMCs	●
Kim and Chae (2004)	Pig	14 and 28 DPI	RT-PCR	Tracheobronchial lymph nodes slightly upregulated in CoI pig	Not different in IPCV2 groups

IL-8

References	Experimental model	Measure time	Measure methods	Measure point	Profile
Goodman *et al.* (1992)	Pig	1 week suffering a progression and respiratory disorders	RT-PCR	Inguinal, bronchial lymph nodes tonsils, spleen and thymus	No difference
Kekarainen *et al.* (2008)	Pig	Not mentioned in mRNA level	Semi-quantitative PT-PCR for mRNA levels	Blood	Increase
Keene and Forman (1982)	Swine AMs	18-108HPI	ELISA	Culture supernatants	Increased in 18,36 and 54 HPI
Allen *et al.* (1990)	Pig	1 DPI	Capture ELISAs	Serum	Increased
Kennedy *et al.* (2000)	Pig	No mentioned	RT-PCR	Lung	Increase
Kim and Chae (2004)	Pig	14 and 28 DPI	RT-PCR	tracheobronchial lymph nodes	Increase

IL-10

References	Experimental model	Measure time	Measure methods	Measure point	Profile
Goodman *et al.* (1992)	Pig	1 week suffering a progression and respiratory disorders	RT-PCR	Inguinal, bronchial lymph nodes tonsils, spleen and thymus	Δ
Kekarainen *et al.* (2008)	Pig	Not mentioned in mRNA level	Semi-quantitative PT-PCR for mRNA levels	Blood	Increase
Hou *et al.* (1994)	Pig	Not mentioned in mRNA level	Semi-quantitative PT-PCR for mRNA levels	Blood	Decrease
Naugler and Karin (2008)	Pig	0, 3, 7, 10, 14, 21, 28 and 35 DPI	Bioassay	Serum	Increase from 10 DPI
McGaha *et al.* (2003)	PBMCs from PRV-immunized pigs	Not mentioned	ELISA	Culture supernatants	Increase
Smith *et al.* (1997)	Pig	Throughout 10 weeks post-infection	ELISA	Blood	Increase
Allen *et al.* (1990)	PBMCs from PCV2 infection	Not mentioned	capture ELISAs	Cell culture supernatants	Increase
Meerts *et al.* (2005	PBMCs from PCV2/PRRSV or PCV2 infected piglets	0, 7, 14, 21, 28 and 42 DPI	Quantitative	RT-PCR PBMCs	Increase
Kennedy *et al.* (2000)	Pig	No mentioned	RT-PCR	Lung	No difference
Kim and Chae (2004)	Pig	14 and 28 DPI	RT-PCR	Tracheobronchial lymph nodes	No difference

IL-12

References	Experimental model	Measure time	Measure methods	Measure point	Profile
Goodman *et al.* (1992)	Pig	1 week suffering a progression and respiratory disorders	RT-PCR	Inguinal, bronchial lymph nodes tonsils, spleen and thymus	▲
Kekarainen *et al.* (2008)	Pig	not mentioned in mRNA level	Semiquantitative PT-PCR for mRNA levels	Blood	No difference
		Short-time after stimulating in protein level	FCICD for protein level	Intracellular of PBMCs	

Table 5: Continue

	IL-12				
References	Experimental model	Measure time	Measure methods	Measure point	Profile
Hou *et al.* (1994)	Pig	Not mentioned in mRNA level	Semiquantitative RT-PCR for mRNA levels	Blood	Increase in protein level
		Short-time after stimulating in protein level	FCICD for protein level	Intracellular of PBMCs	
Grau *et al.* (2001)	Mice	7 and 35 DPI with naked DNAs	Bio-Plex cytokines assays	Blood	Decreased with ORF 2, 3 and 4 vaccination on DPI 7
Meerts *et al.* (2005)	PBMCs from PCV2/PRRSV or PCV2 infected piglets	0, 7, 14, 21, 28 and 42 DPI	Quantitative RT-PCR	PBMCs	Increase
Kim and Chae (2004)	Pig	14 and 28 days post infection	RT-PCR	Tracheobronchial lymph nodes	Decrease
McGaha *et al.* (2003)	PBMCs from PRV-immunized pigs	Not mentioned	ELISA	Culture supernatants	Decrease

	IFN-γ				
References	Experimental model	Measure time	Measure methods	Measure point	Profile
Goodman *et al.* (1992)	Pig	1 week suffering a progression and respiratory disorders	RT-PCR	Inguinal, bronchial lymph nodes	○
Kekarainen *et al.* (2008)	Pig	Not mentioned in mRNA level	Semiquantitative PT-PCR for mRNA levels	Blood slightly	Increased in RNA
		Short-time after stimulating in protein level	FCICD for protein level	Intracellular of PBMCs	Slightly decreased in protein
Grau *et al.* (2001)	Pig	Not mentioned in mRNA level	Semiquantitative PT-PCR for mRNA levels	Blood	Increase
		Short-time after stimulating in protein level	FCICD for protein level	Intracellular of PBMCs	
McGaha *et al.* (2003)	PBMcs from PRV-immunized pigs	Not mentioned	ELISA	Culture supernatants	Decrease
Grau *et al.* (2001)	Mice	7 and 35 DPI with naked DNAs	Bio-Plex cytokines assays	Blood	Increased with ORF1 vaccination on DPI 35
Allen *et al.* (1990)	PBMCs from PCV2 infection	Not mentioned	Capture ELISAs cell	Culture supernatants	No induction
Meerts *et al.* (2005)	PBMCs from PCV2/PRRSV or PCV2 infected piglets	0, 7, 14, 21, 28 and 42 DPI	Quantitative RT-PCR	PBMCs	Increase
Kennedy *et al.* (2000)	Pig	No mentioned	RT-PCR	Lung	Weakly expression
Kim and Chae (2004)	Pig	14 and 28 DPI	RT-PCR	Tracheobronchial lymph nodes	Decrease

	IFN-α				
References	Experimental model	Measure time	Measure methods	Measure point	Profile
Naugler and Karin (2008)	Pig	0, 3, 7, 10, 14, 21, 28 and 35 DPI	Bioassay	Serum	No difference
McGaha *et al.* (2003)	PBMCs from PRV-immunized pigs	Not mentioned	ELISA	Culture supernatants	Decrease
Allen *et al.* (1990)	PBMCs from PCV2 infection	Not mentioned	Capture ELISAs	Cell culture supernatants	Can detect
Kim and Chae (2004)	Pig	14 and 28 DPI	RT-PCR	Tracheobronchial lymph nodes	Decrease

	TNF-α				
References	Experimental model	Measure time	Measure methods	Measure point	Profile
Kekarainen *et al.* (2008)	Pig	Not mentioned in mRNA level	Semi-quantitative PT-PCR for mRNA levels	Blood	Slightly increased in RNA
		Short-time after stimulating in protein level	FCICD for protein level	Intracellular of PBMCs	No difference in protein
Hou *et al.* (1994)		Short-time after stimulating in protein level	FCICD for protein level	Intracellular of PBMCs	Increased in PDNS pigs, no difference in PMWS pigs

Table 5: Continue

	IL-12				
References	Experimental model	Measure time	Measure methods	Measure point	Profile
This study	Porcine alveolar macrophages	0, 2, 8, 12, 16, 24 and 48 HPI	ELISA	Cell culture supernatants	Increase
	Pigs	0, 4, 7, 10, 14, 17, 21, 24, 28, 31 and 35 DPI	ELISA	Serum	Increase
Keene and Forman (1982)	Swine AMs	18-108HPI	ELISA	Culture supernatants	Increased
Grau *et al.* (2001)	Mice	7 and 35 DPI with naked DNAs	Bio-Plex cytokines assays	Blood	Increased with ORF3 vaccination on DPI 7
Allen *et al.* (1990)	PBMCs from PCV2 infection	Not mentioned	Capture ELISAs	Cell culture supernatants	Can detect sporadically
Meerts *et al.* (2005)	PBMCs from PCV2/PRRSV or PCV2 infected piglets	0, 7, 14, 21, 28 and 42 DPI	Quantitative RT-PCR	PBMCs	Increase

#: IL-1α mRNA and IL-1β level were notably increased in the PMWS affected pigs; @: IL-1α mRNA level significantly elevated but no notable differences in protein expressions for IL-1β be found; **: No difference was found in bronchial lymph nodes, tonsils and thymus, decreased in spleen and inguinal lymph nodes; *: Decreased in mRNA level; increased in protein level; ⊠: IL-2 mRNA level decreased in PRRSV/PCV2 co-infection group from 7 DPI, no difference in PCV2 infection group compared the control group; §: IL-4 expression level decreased in tonsils and inguinal lymph nodes; No difference in bronchial lymph nodes, spleen and thymus of the pigs infected with PCV2; ″: No difference about IL-4 mRNA level, IL-4 protein level decreased; №: IL-4 mRNA level was notably decreased in both PDNS and PMWS pigs; IL-4 protein level decreased in PMWS pigs and increased in PDNS pigs; ○: IL-4 mRNA level had significant reduced in PRRSV/PCV2 co-infected piglets at 7, 14, 21 and 42 days post-infection and in PCV2 piglets at 7 days post-infection; ●: IL-6 mRNA level had significant increased in PCV2 infected piglets at 7, 14, 21, 28 and 42 days post-infection, in PCV2/PRRSV co-infection piglets at 7 and 42 days post-infection; Δ: No difference in bronchial lymph nodes, tonsils and spleen; increased in thymus; decreased in inguinal lymph nodes; ▲: No difference in bronchial lymph nodes, tonsils and thymus; decreased in spleen and inguinal lymph nodes; ○: No difference in bronchial lymph nodes, spleen and thymus; increased in tonsils; decreased in inguinal lymph nodes; FCICD: Flow Cytometric Intracellular Cytokine Detection, PRRSV: Porcine Respiratory and Reproductive Syndrome virus; PCV2: Porcine Circovirus Type 2; PMWS: Post-weaning Multisystemic Wasting Syndrome; PDNS: Porcine Dermatitis and Nephropathy Syndrome; DPI: Days Post Infection; PBMCs: Peripheral Blood Mononuclear Cells; ELISA: Enzyme Linked Immunosorbent Assay, ORF: Open Reading Frame; AM: Alveolar Macrophage; HPI: Hours Post Incobation; PRV: Pseudorabies Virus

As an immunosuppressive disease, the distinctive histopathological findings are lymphocyte depletion and histiocytic infiltration of lymphoid tissues (clinical observation). As reported, PCV2 genome or antigen could be detected in several cell types such as macrophage/ monocyte lineage cells, dendritic cells, epithelial cells, hepatocytes, enterocytes, vascular endothelium and lymphocytes (Shibahara *et al.*, 2000; Darwich *et al.*, 2003a, b). The mechanisms of immunosuppression in PCV2 infection were described by lymphocyte depletion, interfering with antigen presentation and the induction of apoptosis in immune system for its main target cells were thought to be the monocyte/macrophage lineage cells and other antigen-presenting cells (Kennedy *et al.*, 2000). Moreover, recent studies had suggested that lymphocyte-like cells may be an important cell populations that supported early PCV2 replication whereas monocytes may be the site for PCV2 persistence in the infected host (Yu *et al.*, 2007). So, these could explain that PCV2 infection could down-regulated IL-2, IL-4 and IL-12 expression but up-regulated proinflammatory mediators (IL-8, IL-1 and TNF-γ) and other chemokines expression. Furthermore, IL-1β was known to induce IL6 thus it was not unexpected that IL6 mRNA levels would not be elevated.

Other research indicated that PCV2 infection could impair Natural Interferon Producing Cells (NIPCs) activity (Vincent *et al.*, 2005, 2007). This was enough to explain PCV2 infection decreased IFN-α level. The number of IL-10 producing cells was higher in all PMWS animals compared to control pigs and not infected with PCV2 (Crisci *et al.*, 2010). This could explain IL-10 was increased in PCV2 infection. Furthermore, IL-10 was able to inhibit the activity of Th1 cells, Natural Killer (NK) cells and macrophages (Couper *et al.*, 2008) thus it is not unexpected that IL-2, IL-4, IL-12 and IFN-α levels would not be decreased. The IFN-γ, it should be decreased in PCV2 infection for this infection could suppress Th1 responses and increase IL-10 level. Conversely, the IFN-γ level was increased in the PCV2 infection for it had been reported to enhance PCV2 infection and replication *in vitro* (Meerts *et al.*, 2005).

However, the reports in this field in different studies differed and this phenomenon usually happed in the lab. Why the results about the cytokines profile with PCV2 infection in different reports were mixed? Listed reasons could explain this confused phenomenon. Different animal models which contained the age, gender, nutrition and health. For example, Shi *et al.* (2010) found that many cytokines profile (i.e., IL-2) were differ in PCV2 infected piglets and PCV2/PRRSV co-infected piglets. The time when resaerchers measured differed. Many cytokines were shorted live once induction and had their optimal detected time. That's means once we missed the optimal time, researchers would not got an exact result. For example, Chang *et al.* (2006) found that IL-8 in culture

supernatants of PCV2-inoculated swine AMs was higher than control group in 18, 36 and 54 h post inoculation but no difference was found beyond that. Specimen researchers choose also played a role in the results. For example, Darwich *et al*. (2003b) reported that no difference about IFN-γ expression level in bronchial lymph nodes, spleen and thymus; increased IFN-γ expression level in tonsils; decreased IFN-γ expression in inguinal lymph nodes were found in the pigs infected with PCV2. Other parameters such as measure methods, control models and statistical methods also directly affect the conclusions.

CONCLUSION

All this review presented an exact cytokines profile with PCV2 infection. The further researches are how to use this knowledge to control PCV2 infection.

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